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STUDIES ON *RHIZOCTONIA SOLANI* KÜHN.

III. RACIAL DIFFERENCES IN PATHOGENICITY[1]

BY G. B. SANFORD[2]

Abstract

Pathogenicity tests on potato stems were made of 133 isolates of *Rhizoctonia Solani* Kühn. Of these, 114 were from random sclerotia on random tubers from four fields, 13 from lesions on potato stems, and 8 from single basidiospores. A number of tests were made in the laboratory at 17° and 23° C., in two contrasting types of artificially infested, unsterilized, virgin soil, which was maintained at optimum moisture content for disease expression.

More of the isolates were pathogenic in the infertile podsol soil than in the fertile black loam. Eighteen per cent of the isolates were of virulent rank in the latter soil, in contrast to 34% of them in the former one.

Indications from the study were that, under average soil conditions, approximately 20 to 50% of the isolates of *R. Solani* from sclerotia on random tubers may be assigned to the zero and marginal classes of pathogenic rank. The data also indicated that certain isolates were inherently very deficient in pathogenicity to potato stems, while others characteristically possess a high degree of virulence. Thus, with regard to the effect of soil type and racial differences in pathogenicity, it would appear that the results of this study help to explain why the stems of a high percentage of plants from sclerotia-infested sets often escape with little or no infection under field conditions.

In a previous study (14), involving 34 tests of an experiment made in different fields, an average of about 42% of the 3,400 plants, which grew from sets heavily infested with sclerotia of *Rhizoctonia Solani*, had lesions on the stems at the end of six weeks. But when a deduction was made for the infection from the soil, an average of only approximately 29% of the plants had lesions of any size, attributable to the sclerotia on the sets. In only four cases did the sclerotia on the sets augment the percentage of infected plants by more than 50%. In approximately one-third of the tests the indicated increase fell below 20%. Finally, in those soils apparently having a relatively light natural infestation, the increases produced by the sclerotia-infested sets were extremely variable and certainly less severe than what might be expected. The following paired results, which are taken from Table I (14), will illustrate this: 98-81; 95-25; 99-32; 96-24; 91-31; 86-50; 80-15; 90-21; 95-37 and 81-42. In each case the first figure given represents the percentage of plants with clean stems from clean treated sets, and the second one the percentage of infected plants for which the sclerotia-infested sets were responsible.

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The unexpectedly low effectiveness of the sclerotia on the sets was not explained by the data available. However, it seemed that one of several contributing factors might be the presence of a large proportion of sclerotia of marginal pathogenicity.

This paper will be confined to a study of the relative disease-producing ability on potato stems of 133 isolates of *R. Solani*, as obtained from the potato plant in six different fields in the Edmonton district.

Literature Review

Braun (1), in 1930, reviewed the literature relating to racial specialization in *R. Solani*, as indicated by morphological, cultural and host reaction phenomena. Therefore, only a few typical references are necessary here.

Matsumoto (6) secured, with Isolate P, infection of *Solanum tuberosum*, *S. melongena*, *Lactuca sativa*, *Phaseolus vulgaris* and *P. lunatus*. He states: "The pathogenic capacity of the strains is always manifest when inoculation is made on plants belonging to the same species of host as that from which the culture originated".

LeClerg (4), 1934, concluded that rhizoctonia root rot of sugar beets in field culture is probably caused by strains distinct pathogenically from those which attack the potato plant; also that groups of isolates from the lesions on stems of potato, and those from the diseased tissue of sugar beets were about equally destructive in damping off alfalfa seedlings.

Peltier (9), 1916, concluded that the many isolates which he obtained from a wide range of hosts were able to attack the same species of plant, and that no marked host specialization was noted in any of his isolates.

With regard to racial characteristics other than pathogenicity, Matsumoto (6), 1921, found that certain isolates differed from others in enzyme activity and in ability to utilize potassium nitrate. Edwards and Newton (2), 1937, reported that isolates from the same or from different hosts might express either different or similar enzyme activity. They did not report the relation of enzyme activity of several isolates to their relative virulence on a given host.

Racial differences in growth, as affected by temperature, have been reported by Matsumoto (6), Monteith and Dahl (7), LeClerg (4), Rosenbaum and Shapovalov (12) and others. For growth, the most common optimum appears to have been between 22° and 25°, while for a relatively smaller number of isolates it was somewhat higher than 25° C., or slightly lower than 22° C. Richards (11) obtained growth from 4.6° to 32.5° C. However, for greatest pathogenicity to potato, bean and pea, the optimum was approximately 18° C., while for the isolates pathogenic to the sugar beet root, LeClerg (4) concluded that a soil temperature of approximately 25° to 33° C. was best. For carnation rot Peltier (9) found temperatures above 88° F. most favorable.

Certain other factors are said to affect the virulence of the pathogen. Rabinovitz-Sereni (10), Gratz (3), Weber (15), Samuel and Garrett (13), and

others have found the optimum hydrogen ion concentration for growth to lie between pH 6.5 and 7. The effect of age and condition of culture have been discussed by several workers, but without general agreement as to whether a young culture is more virulent than an old one. Wellensiek (16) found the virulence of a pure culture unchanged after eight years. Peltier (9) concluded that a soil moisture content either too low or too high for best growth of the carnation plant favored the disease, if the temperature was above 88° F. Müller (8) reported that rhizoctonia injury to potato was increased in a wet soil, while Martin (5) observed that it decreased as the soil moisture content increased.

With regard to soil type Müller (8) found that the vegetative growth of *R. Solani* was abundant and rapid in a rich loam, but restricted and slow in a sandy, infertile soil. He argued that the former type of soil, by providing a more abundant and rapid growth of the pathogen, would be more favorable to development of the disease than the latter type.

Experimental Methods

Numerous preliminary trials were made to obtain a satisfactory method to test the disease-producing ability of the isolates. Attention was given to type of soil, soil temperature, soil moisture, method of infesting the soil, and the time required for an adequate test.

Soil Type

Two contrasting types of virgin soil were chosen, and each used with its natural microflora, except for the addition of a small fraction of soil inoculum. One was the black organic loam (Type I) common to the park area of Alberta, and the other a white podsol (Type II), characteristic of the wooded areas of certain parts of Alberta. Wyatt and Newton (17) have provided a description of the physical properties, and also a chemical analysis of the A, or cultivated, horizon of each of the two types mentioned. Following are the data: Type I, nitrogen 0.53, phosphorus 0.10, calcium 0.94, magnesium, 0.50, soil reaction pH 6.4; Type II, nitrogen 0.09; phosphorus, 0.06, calcium 0.55, magnesium 0.33, soil reaction pH 6.2.

Soil Moisture and Temperature

At the beginning of the test the artificially infested soil was made up to approximately 29% of its moisture-holding capacity for Type I, and 27% for Type II. This moisture content was what might be considered optimum for each soil. Water was not added during the test. Under the conditions of the experiment the moisture lost during the test varied between 1 and 1.5%.

Two soil temperatures were used for testing each isolate, namely 16°–17° C., and 23°–25° C., but usually 17° and 23° C.

The Experiment

Each isolate was increased in steam sterilized soil without additional nutrient. This pure soil inoculum was mixed in the proportion of one part of inoculum to 15 parts of natural soil, made up to the required moisture content.

A potato set was placed on a small amount of this soil at the bottom of each one-litre Erlenmeyer flask, and covered to a depth of approximately six inches. Cotton plugs were inserted in the flasks. The flasks were then placed at the required temperature for 21 days.

The variety used was Early Ohio, which is known to be very susceptible to rhizoctonia stem canker. The tubers from which each of the sets was cut were from one lot kept in cold storage until required. Fifteen sets were used for each test.

At the end of 21 days, when the sprouts had emerged from the soil, a numerical disease rating was given for each unit, and the length of the sprout measured. Also, the massing of the hyphae and the formation of sclerotia on the stem or set, if any, were recorded. The soil moisture was determined at this time. The infections, expressed in percentage, for each isolate for each type of soil, and for the two temperature series, are listed in Table I.

The Isolates

The six groups of isolates tested were secured from six different sources. In four of the six groups (A, B, C and D) each isolate was obtained from one sclerotium on one tuber, and each tuber was taken from a separate and random hill in the potato field. In another case, they were taken from rhizoctonia lesions on different potato stems. Definitely monosporus cultures from basidiospores of the *Corticium* stage produced on potato stems comprised Group F. In isolating these monosporous cultures it was absolutely necessary to decide by careful microscopic examination whether the growth on nutrient agar below the suspended hymenial structure was from definite basidiospores or merely from hyphal fragments which were frequently set free.

With the exception of the isolates from the basidiospores, any of the other isolates might be, in a sense, mass cultures, even after repeated transfer in pure culture. Moreover, isolates from sclerotia on tubers, as well as many of them from stem lesions, are merely representative of the fungus as distributed in the soil of a given field at the time the sclerotia formed, and not necessarily pathogenic forms. The isolates, as indicated, were considered more suitable for the immediate purpose of the study than hyphal-tip isolates would be.

Results

The experimental results, which are listed in Table I, indicate the relative disease-producing ability on potato stems, during 21 days, of each of the 133 isolates tested. Detailed records prove that throughout these tests there was always adequate growth, on the potato stems, of hyphae of all isolates to produce disease. It is important to observe in Table I the percentage of stems which escaped attack, since this seems to be closely related to the pathogenic ability of an isolate. For the convenience of the reader, the data in Table I have been summarized in Table II to indicate the percentage of isolates pathogenic, and the relative severity of disease in each of the two contrasting soil types. In Table III the data are again arranged to show the percentage of isolates of each series according to degree of virulence. The illustrations

in Plate I indicate the inherent ability of different isolates to produce severe, medium, light, or no disease.

Effect of Soil Type

A comparison of the data in Table I, and also in Table II, leaves no doubt that in the first test, at least, a distinctly higher proportion of isolates were pathogenic in Soil II than in Soil I, and also that, in general, the isolates

TABLE I

PATHOGENICITY OF 133 ISOLATES OF *Rhizoctonia Solani* FROM SIX DIFFERENT SOURCES, AS INDICATED ON POTATO STEMS IN TWO CONSTRASTING TYPES OF SOIL AT APPROXIMATELY 17° C.

No.	Soil I				Soil II	
	17°		23°		17°	
	Rt.†	*	Rt.†	*	Rt.†	*
(Series A)‡						
1	41	25	66	0	51	6
2	43	12	32	31	70	0
3	33	25	7	81	36	19
4	11	62	12	62	0	100
5	0	100	0	100	13	44
6	28	6	18	31	32	31
7	7	75	19	25	16	75
8	27	44	31	38	54	6
9	0	100	0	100	8	81
10	0	100	27	31	0	100
11	18	31	7	81	16	62
12	0	100	2	88	2	75
13	16	62	44	6	53	6
14	8	81	28	31	60	0
15	26	50	32	31	60	0
16	0	100	0	100	2	94
17	0	100	0	100	26	19
18	0	100	0	100	35	19
19	1	88	0	100	27	44
20	45	25	4	19	20	44
21	1	81	0	100	39	12
22	0	100	0	100	50	6
23	0	100	0	100	28	44
24	0	100	0	100	28	19
25	0	100	0	100	31	0
(Series B)‡						
1	36	31	10	81	60	0
2	32	31	24	50	62	0
3	2	81	0	100	46	6
4	44	19	26	50	61	6
5	0	100	0	100	5	81
6	0	100	0	100	11	81
7	10	62	10	56	67	0
8	0	100	0	100	10	57
9	5	81	4	88	27	56
10	30	44	10	75	45	31
11	0	100	0	100	12	94
12	0	100	0	100	11	69
13	0	100	0	100	31	31
14	0	100	0	100	25	38
15	0	100	0	100	37	31
16	0	100	0	100	15	75
17	6	81	6	94	37	38
18	0	100	0	100	10	81
19	0	100	0	100	11	75
20	0	100	0	100	5	88
21	0	100	0	100	3	94
22	0	100	0	100	49	6
23	0	100	0	100	47	12
24	0	100	0	100	22	44
25	0	100	0	100	30	50
(Series C)‡						
1	3	75			16	69
2	0	100			80	0
3	0	100			41	31
4	4	75			21	56
5	0	100			16	62
6	0	100			0	100
7	0	100			2	88
8	0	100			12	62
9	0	100			1	88
10	0	100			4	69
11	0	100			0	100
12	0	100			36	19
13	0	100			70	0
14	10	81			11	69
15	7	81			0	100
16	0	100			0	100
17	0	100			12	75
18	0	100			25	50
19	1	94			13	69
20	0	100			36	31
21	0	100			1	56
22	0	100			13	50
23	2	88			14	75
24	0	100			12	44
25	0	100			8	44

† *Disease rating %.* * *Per cent plants not lesioned.*

‡ *A, B and C, from random sclerotia on potatoes in field.*

TABLE I—*Concluded*

PATHOGENICITY OF 133 ISOLATES OF *Rhizoctonia Solani* FROM SIX DIFFERENT SOURCES, AS INDICATED ON POTATO STEMS IN TWO CONTRASTING TYPES OF SOIL AT APPROXIMATELY 17° C.—*Concluded*

No.	Soil I				Soil II			
	Test 1		Test 2		Test 1		Test 2§	
	Rt.†	*	Rt.†	*	Rt.†	*	Rt.†	*
(Series D)‡								
1	0	100	18	70	54	10	30	50
2	0	100	0	100	0	100	0	100
3	52	0	70	0	70	0	63	10
4	0	100	0	100	0	100	0	100
6	0	100	20	10	3	90	0	100
7	0	100	46	50	0	100	0	100
8	44	0	49	10	62	0	0	100
10	0	100	6	80	0	100	0	100
11	0	100	0	100	0	100	0	100
12	2	90	0	100	51	20	60	40
14	0	100	0	100	10	70	16	70
15	0	100	0	100	0	100	1	100
16	0	100	2	80	0	100	0	100
17	5	90	10	60	0	100	0	100
18	0	100	0	100	7	80	5	90
19	0	100	1	80	0	100	14	80
20	54	0	45	20	60	0	16	0
21	6	0	7	70	9	90	20	70
22	0	100	0	100	0	100	1	100
24	7	80	60	0	40	30	60	10
25	9	60	60	20	30	40	60	0
26	20	0	0	100	10	80	0	100
28	15	20	18	40	60	0	27	60
29	0	100	0	100	0	100	0	100
30	0	100	13	70	0	100	2	90
31	52	0	55	0	51	10	45	20
35	30	0	30	40	5	80	30	30
36	0	100	0	100	0	100	0	100
38	30	0	50	0	40	20	40	10
39	0	90	32	40	3	60	6	90
42	0	100	31	40	5	80	20	60
43	0	100	50	0	0	100	0	100
44	0	100	4	90	0	100	0	100
45	0	100	3	90	0	100	0	100
(Series D—*Concluded*)‡								
47	0	100	0	100	0	100	1	70
48	0	100	0	100	0	100	0	100
49	8	0	0	100	0	100	0	100
(Series E)‡								
70	0	100	0	100	40	30	0	0
71	0	100	0	100	0	100	0	0
72	18	0	8	80	0	100	0	0
73	0	100	0	100	3	40	8	90
74	0	100	0	100	0	100	0	0
75	0	100	0	100	0	100	0	0
76	40	30	50	10	60	0	55	10
77	0	100	34	40	45	20	17	60
81	0	100	0	100	0	100	0	0
82	0	100	0	100	6	90	25	40
83	0	100	0	100	0	100	0	0
85	0	100	5	80	0	100	0	0
86	0	100	0	100	0	100	0	0
(Series F)‡								
105	0	100	0	100	0	100	0	100
106	13	80	11	80	46	20	10	70
107	0	100	0	100	30	50	0	100
108	0	100	56	0	63	0	0	100
109	0	100	0	100	12	70	0	100
110	1	90	34	40	40	30	0	100
111	0	100	0	100	37	30	0	100
112	2	90	0	100	30	50	0	100

† *Disease rating %.*

‡ *A, B and C, from random sclerotia on potatoes in field. D, from random sclerotia on potatoes in field; E, from random lesions on potato stems in field; F, isolates from single basidiospores.*

§ *Second test made by replanting soil of first test.*

* *Per cent plants not lesioned.*

produced more disease in the former soil. The percentage of isolates pathogenic in the four tests of all series in Soil I was 34, while for Soil II it was 73. For severity of disease the corresponding figures are 6% and 22%, respectively. The detailed data given in Table I confirm the trend just indicated. This general tendency has also been observed in a number of other experiments not reported here. Owing to the operation of certain factors, which apparently

are not significantly affected by either soil temperature or soil moisture content, the disease expression in either soil may be slight or great. Whatever the cause may be, all isolates appear to respond in a given test in the same general direction of disease expression. Also, as a rule, the trend is indicated both by the percentage of isolates pathogenic and by the numerical disease rating. For example (Tables I and II), when the two soils were replanted immediately after the first test had been completed, the difference just indicated occurred in the second test in both soils. In this second test a number of isolates which expressed slight or no pathogenicity in Soil I in the first test produced more disease, and in Soil II certain erstwhile marginally pathogenic isolates failed to produce disease. The percentages of the isolates pathogenic in the first tests in Series D, E and F for Soil I were 38, 8 and 38, and for the second test 62, 25 and 38; for the first test in Soil II, 49, 33 and 87, and for the second test 51, 25 and 13.

Percentage of Isolates which Produced Disease

The ability of an isolate to produce disease was judged by its performance in one or more pathogenicity tests which were made in two contrasting types of soil, and some of them at two temperatures, *viz.*, 17° and 23° C. For example, isolates Nos. 1–50, inclusive, received three tests; Nos. 51–75, inclusive, two tests; and the remaining 57 isolates four tests at 17° C. The data in Table I emphasize that it would be very unsafe to rely on one test, no matter how well it was replicated or how favorably the soil temperature and moisture were maintained. Apparently there are certain isolates which are fairly constant in producing a substantial, although often variable, amount of disease, while others tend to be marginal, regardless of soil type, and, finally, certain ones appear to lack the ability to cause disease, even under a fairly wide range of

TABLE II

AN ANALYSIS OF THE PATHOGENICITY OF 133 ISOLATES OF *Rhizoctonia Solani* ON POTATO IN TWO CONTRASTING SOIL TYPES, ACCORDING TO SOURCE OF ISOLATE AND AVERAGE DISEASE RATING

Series	%* isolates producing disease						Av. disease rating % in Test 1		
	17° C.				23° C.	†			
	Soil I		Soil II		Soil I		Soil I		Soil II
	Test 1	Test 2	Test 1	Test 2	Test 1		17° C.	23° C.	17° C.
A	56		92		56	100	12	14	30
B	32		100		28	100	7	4	30
C	24		84			88	1		17
D	38	62	49	51		84	9		15
E	8	25	33	25		54	4		12
F	38	38	87	13		88	2		32
	34‡	50§	73‡	40§		86‡	6‡		22‡

* *To nearest decimal.*
† *Pathogenic in at least one of several tests.*
‡ *Percentage of 133 isolates.*
§ *Percentage of 58 isolates, as given in both soils of Test 1, replanted.*

conditions. All isolates, whether usually very pathogenic or those of marginal nature, evidenced more or less erratic behavior, and although it is not entirely evident from these data, there is indication that the isolates of marginal pathogenicity are the most erratic.

TABLE III

AN ANALYSIS OF THE PATHOGENICITY OF 133 ISOLATES OF *Rhizoctonia Solani* AS EXPRESSED ON POTATO STEMS. THE PERCENTAGE OF ISOLATES IN EACH VIRULENCE CLASS IS GIVEN FOR EACH OF THE SIX SOURCES IN TWO SOIL TYPES

Series	Soil I				Soil II			
	Zero	Marginal	Intermediate	Virulent	Zero	Marginal	Intermediate	Virulent
	0	1–15	16–30	31+	0	1–15	16–30	31+
A	36	16	24	24	8	16	28	48
B	68	16	4	12	0	36	20	44
C	76	24	0	0	16	48	16	20
D	30	27	13	30	38	24	11	27
E	69	8	8	15	61	8	8	23
F	50	25	0	25	13	0	37	50
*	52	20	10	18	22	26	18	34

* *% of 133 isolates.*

The data in Table II illustrate in summary form the variability in pathogenicity which occurred between the first and second tests of the isolates, and also the effect which a different soil type may exert on it. If a disease rating of form 1–15%, inclusive, represents disease-producing ability of marginal rank, 16–30%, intermediate rank, and 31+%, virulent rank, the 133 isolates of the five series can be classified on the basis of performance in all tests given them in both soil types (Table III). Thus, for Soil I, 52% of the 133 isolates produced no disease, while 20%, 10% and 18% of them were of marginal, intermediate and virulent rank, respectively; while for Soil II, only 22% of them failed to produce disease, and the corresponding percentages were 26, 18 and 34, respectively. The percentage of isolates of virulent rank in Soil I was 18, and in Soil II, 34. Fourteen per cent of the isolates produced no disease in any test made in either soil. The practical bearing to the rhizoctonia disease problem of a relatively high percentage of isolates having little or no ability to produce disease, and a relatively low percentage of them having virulent rank, will be referred to later.

Source of Isolate

One purpose of this study was to learn whether isolates, as derived at random from one field or source, may be more virulent than those from another. A comparison of the relative disease-producing ability of the isolates from the five series is available in Tables II and III. The percentage of isolates in each series, which produced disease in one or more tests in both soils, is listed in Column 7 of Table II. The 25 isolates each of series A and B

all produced disease, while 88% of the 25 in Series C, 84% of the 37 in D, 54% of the 13 in E, and 88% of the 8 isolates in Series F produced more or less disease. Perhaps only in Series A, B, C and D was the number of isolates adequate, because it is evident from these studies that definite conclusions with regard to pathogenic ability of a group of isolates cannot be made without testing a fairly large number, and repeating the test under different conditions. Yet, one cannot ignore the apparently significant trends in the data which suggest that not only may there be a different percentage of the isolates from one source pathogenic than from another, but that there may also be a difference in the proportion of isolates of virulent rank to those of intermediate or marginal rank in each case. These trends are also illustrated by the data in Table III, where, under a slightly different arrangement of the isolates of each series according to their disease-producing ability, rather marked differences appear. For example, in Series C, Soil I, none of the isolates ranked as virulent or even as intermediate, and a similar tendency also occurred again in Soil II. Also, in Series A and F, of Soils I and II, there were correspondingly more isolates of virulent rank than in the other series. Series F (Soil II), having 50% of the isolates of virulent rank and 37% of intermediate rank, is of special interest, because these isolates were from single basidiospores of the *Corticium* stage. Of the 13 isolates of Series E (from lesions on potato stems) 69% failed to produce disease in Soil I, and 61% in Soil II. The corresponding percentages for Series A, B, C, D and F in Soil I are 36, 68, 76, 30 and 50, respectively; and in Soil II, 8, 0, 16, 38 and 13, respectively. The obvious conclusion from these data is that without a thorough test one cannot assume that an isolate from a lesion is pathogenic merely because it had its origin there. But, apart from this consideration, there does appear the possibility that, in general, the sclerotia on tubers from one field may be less able to produce disease than those from another field. The situation with regard to the relative disease-producing ability of isolates obtained from basidiospores as compared to that of those from sclerotia cannot be decided from the data given. However, it was proved that spores from the *Corticium* stage may yield a very high percentage of virulent isolates, and also, like cultures from sclerotia, may yield isolates which ordinarily fail to produce disease. A study of the relation of the *Corticium* stage to the disease problem appears to merit more careful study than has yet been given to it.

Discussion

The main points of interest emphasized by the data presented are that in nature *R. Solani*, as obtained from the potato plant, commonly consists of many forms or races, which differ widely in ability to produce lesions on potato stems, and that, among other possible factors, this ability may be influenced very definitely by the type of soil in which the test is made. Obviously, these two characteristics have an important relation to the problem of disease control.

No satisfactory explanation is available from the data for the marked differences in disease expression of the isolates in the two soil types. According to Wyatt and Newton (17) the nitrogen in Soil I was approximately six times greater than in Soil II, while the phosphorus, calcium and magnesium were each nearly twice as much. Whether the marked difference in disease response was associated with these differences, or with the dissimilar physical nature and microflora of the two soils is unknown. The data indicate that the sets furnished ample food for the host in both soils during the short period of test. Thus, it seems probable that the difference in disease expressed can be explained by a change in the pathogen in each case, and not by a change in the host. The growth of the isolates was sufficiently abundant in both soils to cause a maximum of disease, if other conditions were favorable. Therefore, contrary to Müller (8), it seems questionable whether the actual relation between either a rapid or an abundant growth of the pathogen and its ability to produce disease is sufficiently well understood to postulate the effect of these factors under natural field or laboratory conditions. Despite the strong and constant tendency in the first planting for less disease in Soil I than in Soil II (Table I), the expression was at times similar in the two soils, or even less in Soil II than in Soil I, although the temperature and moisture factors were the same. Thus, it would appear that certain obscure factors may operate to counteract the usual effect of a given soil type.

The data in Table I show that, ordinarily, a collection of isolates made at random either from sclerotia on tubers, lesions on stems, or from basidiospores in any field, may consist of a surprisingly high percentage which are nonpathogenic or weakly pathogenic, and apparently a relatively low percentage of those which may, under ordinary conditions, be classified as really virulent. Of the 133 isolates tested in this study, those with no pathogenicity, and those of marginal rank in Soil I made up 72% of the total, and 48% when tested in Soil II (Table III). In Soil I, 18% of them exhibited virulent rank, and in Soil II, 34% of them. Thus, it seems that this information helps to explain why, in the 34 field experiments previously reported (14), the stems of an average of approximately 29% of the 3,400 plants from sclerotia-infested sets had no lesions. The fact that there was more than one sclerotium on each set planted might introduce a difficulty in this explanation, especially if the majority of the sclerotia on each set were of different origin. However, if the majority of them were of one origin (or consisted of weakly pathogenic strains) there would be, as these studies indicate, many cases of escape. The additional possible effect of soil type in a field has already been referred to.

The sclerotia on the tubers should be a fairly representative sample, in disease-producing ability, of *R. Solani* as it exists in the soil of the field when the sclerotia are formed. Obviously, sclerotia may be abundant or extremely scarce on the tubers, irrespective of the presence or absence of disease lesions on the plant. If much disease is present in a given field, it is logical that not only have the soil and other conditions been favorable, but that virulent races are relatively abundant. Hence, sclerotia from such a field might tend,

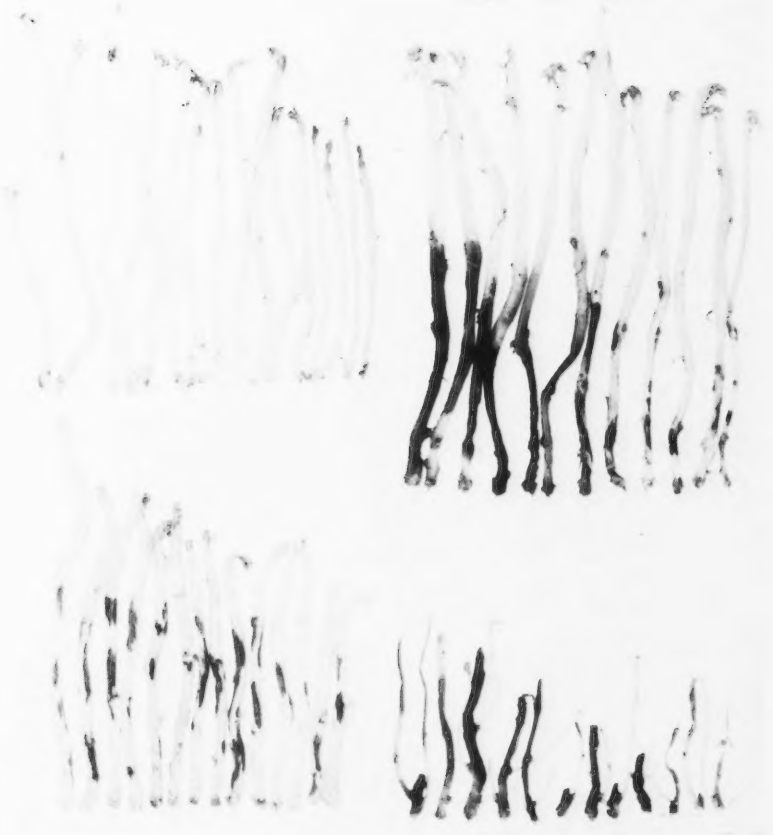

PLATE I. *Each group represents the inherent ability of different isolates of* ***Rhizoctonia Solani*** *to canker potato stems.*

if introduced on untreated tubers to another field, the population of which consisted of relatively few virulent races, to increase permanently in the new field the proportion of virulent to non-virulent forms. The results of this study suggest that the sclerotia from one field may be more dangerous than those from another. For example, the isolates of non-pathogenic and marginal rank from Field C comprise 100% of that group in Soil I, and 64% in Soil II. Of those from Group E (lesions on stems) the corresponding percentages for Soils I and II were 77 and 69. For Groups A and D, the percentages were 52 and 57, respectively, in Soil I, and 24 and 62, respectively, in Soil II. The percentage of the isolates in Groups A and F which were of virulent rank in Soil I, were 24 and 25, respectively, and in Soil II, 48 and 50, respectively.

The data, based on only 10 samples, indicated that a fairly large proportion of the virulent isolates might arise from the *Corticium* stage. In the field, the *Corticium* stage may or may not be associated with diseased plants, or it may be very prevalent in a field, the potato plants of which are particularly free from disease lesions. Consequently, it is an interesting and important question whether the *Corticium* stage tends to augment even temporarily the percentage of virulent forms.

Further, one asks whether some or all of those isolates apparently not pathogenic to potato in these tests could be induced to attack it by reasonable manipulation of the host, the fungus, or the environment. According to Matsumoto (6) all isolates from potato should attack this host, and Peltier (9) concluded that even though the individual isolates from many different species differed in virulence, all were pathogenic to carnation, if the conditions were made favorable enough for the disease. In the present study 14% of the isolates from potato plants appeared unable to attack the potato under very favorable conditions.

In conclusion, the results of this study are of value in that they present a general idea of the relative ability of random isolates of *R. Solani* from the potato plant to attack the host under normal conditions. However, the data do not indicate what proportion of the 133 isolates might attack (excluding the damping off phase) other economic field crops which may follow a severely infested crop of potatoes. If, as LeClerg (4) suggests, certain isolates are rather specific to the sugar beet root, a further analysis of the general problem might reveal much needed information.

Acknowledgment

The assistance of Mr. G. M. Tosh in this study is acknowledged with appreciation.

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STUDIES OF THE TOMATO IN RELATION TO ITS STORAGE

I. A SURVEY OF THE EFFECT OF MATURITY AND SEASON UPON THE RESPIRATION OF GREENHOUSE FRUITS AT 12.5° C.[1]

By E. J. M. Walford[2]

Abstract

Tomatoes were grown in the greenhouse at different seasons of the year, individual fruits were picked at various stages of maturity and continuous records of their respiration obtained at 12.5° C. It was found that the fruits of the late spring and summer went through the customary series of extensive changes in respiration rate as they ripened at the low temperature, and exhibited the lack of durability normal to this fruit. In contrast to this, the fruits of the late autumn, winter and early spring, if picked before the external appearance of red pigment, passed into a stable state in which ripening proceeded with but little change in respiration rate and with greatly enhanced duration of life at 12.5° C.

The tomato fruit has been investigated with a view to storage much less than the dessert fruits, doubtless because so large a portion of this crop is processed by canners. Nevertheless important quantities, and especially the greenhouse crops, are consumed fresh, and the storage properties of this fruit are of importance in its marketing and transportation. In the future we may anticipate that, as with other fruits, the problems of storage will come to influence even culture and harvesting practice.

Experience shows that the tomato's natural lack of durability is not easily overcome by low temperature. The fruit tends to be intolerant of the severe retardation of its ripening processes by temperatures near 0° C., and to break down rapidly upon removal to higher temperatures after quite short exposures to low temperature (9). The consequence is that moderate, compromise temperatures in the vicinity of 10° to 12° C. are most commonly advocated for field-grown tomatoes in temperate regions (1, 9), though somewhat lower temperatures appear to be permissible for tomatoes grown in the tropics (8). Moderate temperatures usually mean short-term storage for the purpose of increasing the flexibility of marketing, rather than for preservation beyond the season.

The outlook for long-term storage is not very encouraging. Yet it is known from farm experience that late tomatoes removed to a suitably cool place in the autumn may be held, ripening gradually and yielding a supply of marketable fruit, well on into the winter. This suggests that the seasonal factors operative during the growth of the fruit may be effective in influencing durability. There is reason to believe also that growth conditions may be so adjusted as to diminish the intolerance of the tomato to low temperature.

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Contribution from the Department of Horticulture, Ontario Agricultural College, Guelph; with the co-operation of the Department of Botany, University of Toronto. Issued as Paper No. 2 of the Committee on Storage and Transport of Food.

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For instance, Barker (1) found that temperatures below 15.5° C. were injurious to English hothouse tomatoes, whilst Wardlaw and McGuire (8) showed that their tropical fruit was better preserved at about 5° C. Kidd and West (6) later discovered that English summer-grown fruit tends to behave in respect of temperature in the manner of the tropical tomatoes, whereas English autumn-grown fruit gave results such as those obtained by Barker. Evidently there is something to be learned about the influence of season upon the properties of the fruit that concern its preservation in the living state.

Consequently, when it was decided in 1934 to begin the study of tomatoes in relation to their storage, it was felt that one of the most useful additions to our knowledge would be attained by systematic examination of the influence of season, and of maturity at the time of picking, upon pertinent aspects of the physiology of the fruit held at some appropriate storage temperature. The temperature adopted was 12.5° C. It was planned to combine with this survey such bulk storage tests as the results of the physiological enquiry might demand.

This project in its two aspects of physiological survey and storage testing is still in progress. The physiological work done by the writer has chiefly taken the form of tracing the respiration of the fruit throughout its life at the storage temperature. This choice was made because the record of respiration is the most generally useful single index of the gradually changing physiological state of the fruit. To be fully significant such a study demands the treatment of single fruits, otherwise individual peculiarities are obscured by statistical effects. This would defeat one main purpose of the enquiry, which is to detect and characterize at least the more frequent physiological types occurring amongst the fruits at all seasons of the year in correlation with the cyclic variation of the conditions of growth.

The present paper gives an account of the respiration at 12.5° C. of individual fruits picked at various stages of maturity from plants grown in the greenhouse at different seasons during the first year of the investigation. This systematic survey was continued for another two years with confirmatory results, so that a much larger number of fruits have actually been under observation than are reported upon in detail in this paper. It happens that the data of the first year, with slight supplementation, illustrate sufficiently the course of respiration in the fruits, and mere recapitulation of substantially similar results could serve no purpose. The individual records will be examined and compared in some detail, since they form an index to the physiological changes occurring in fruits stored at 12.5° C. They are also the criteria by which we distinguish two types of fruit, the distribution of which has been found to be correlated with the seasonal factor. These types will be constantly before us in later papers of this series, which will embody the results of attempts to modify the distribution of the types through the manipulation of growth conditions, the further characterization of the types, and the history in bulk storage of fruits produced by various methods.

Experimental Procedure

Propagation of the Plants and Growth of the Fruit

Tomato plants of Grand Rapids variety were grown in the greenhouse from seed obtained from a commercial seed house. A standard procedure for germination and transplanting was devised, and the soil kept as uniform as possible throughout. The bench soil was a light clay loam with which was incorporated well rotted manure (30 tons per acre) and a 0-12-12 fertilizer (1500 lb. per acre). When the third truss of fruit was forming, weekly side dressings of nitrate of soda were applied (150 to 200 lb. per acre). The plants were set out with 18-inch centres and grown to a single stem. They were supported and allowed to produce seven trusses of fruit.

The mean temperature of the greenhouse varied with the season, but abrupt or violent fluctuation was avoided. The light periodicity normal to the season was not modified in any of the experiments dealt with in this paper. Pests were not serious, but some fumigation was unavoidable and cyanide was employed at least two weeks before picking the fruit. There was no detrimental effect and we are satisfied that any physiological disturbance occasioned at the time of fumigation was well past before respiration experiments began.

Pollination was assisted by tapping the flowers. The setting of the fruit was identified by the ready separation of the corolla from the receptacle and the date of setting was recorded on a tag attached to each young fruit.

Classification of the Fruits

The age of each fruit was known but the growth and physiological development of the individuals vary, so that fruits of the same chronological age are not necessarily in identical physiological states. It was essential, therefore, to consider other indices of development in choosing the experimental fruits. Consequently, a series of classes was established, within the limits of each of which the fruits would be at least roughly comparable physiologically. This classification is given in Table I. The subsequent physiological test confirmed the assignment of fruits to their place in this classification in all but exceptional cases.

Measurement of the Respiratory Carbon Dioxide

Immediately after picking, the fruits were removed to a room at 12.5° C. the calyces were carefully taken off, the fruits weighed, the calyx scars waxed and the fruits re-weighed. A full description of each fruit was recorded. The fruits were then placed singly in glass respiration chambers consisting of two hemispheres sealed together and provided with tubulatures. The air flow was started, and zero time of the respiration record was taken the following morning, approximately 15 hours later. The air was passed through large towers of soda lime and humidified in 7% potash solution before being led into the respiration chambers. From the chambers each stream passed to a Pettenkofer absorption tube containing barium hydroxide. The Pettenkofer tubes were changed periodically and the residual baryta titrated. The

TABLE I

PHYSIOLOGICAL CLASSIFICATION OF TOMATO FRUITS

Physiological stage	Symbol indicating physiological state on respiration records, tables, etc.	Description of the fruit
Early growing-green	I	Immature, growing, hard, dark green and small in size for the variety. Chronological age up to 32 days.
Late growing-green	II	Maturing, hard, green, medium size for the variety. Chronologically 32 to 42 days.
Mature-green	III	Maximum growth, firm, light-green color.
Yellowing	IV	Yellow color appearing on the base, remainder light green.
Yellow-orange	V	Orange-red base and yellow-green calyx.
Three-quarters red	VI	Evenly orange-red.
Full red	VII	Red.

successive absorption periods were of 24 hours duration in the experiments on fruits from Populations I, II and III, and of 12 hours in the others. Mean rates for each interval were computed in terms of cc. of CO_2 per 10 kg. of fruit per hour. These determinations are represented graphically in Figs. 1 to 35.

As the experiment proceeded, notes were kept of the maturation and senescent changes in the fruit. Where the fruit cannot be handled or destroyed, the only available index of ripeness is external color, so that the correlation of respiration rate with ripeness is essentially a correlation with color. Each graph is provided with a chart which indicates the progress of external color change along the time scale of the respiration record.

The Experimental Materials

The fruits used in the survey experiments of the first year were derived from four plantings of tomatoes which are referred to as Populations I to IV. They were picked at various stages of maturity, but owing to limitations of apparatus, each population could not be represented by fruits of every stage. The character of the fruits is indicated in Table II, in which they are assigned to their parent population and classified according to the scheme previously described.

Figs. 37 and 38 summarize the chronological distribution of the periods of growth and of picking for each population and relate them to the factors of light and temperature.

Populations I and II were summer-grown, and since the records indicate that the fruits of the two populations constitute a single series, they are

TABLE II

NUMBERS OF FRUITS OF POPULATIONS I TO IV, IN VARIOUS PHYSIOLOGICAL STAGES

Population	Early growing-green	Late growing-green	Mature-green	Yellowing	Yellow-orange	Three-quarters red	Full red
	I	II	III	IV	V	VI	VII
I			2	2	2	2	3
II	4	3					
III			3	2		3	2
IV	3				2		2

treated together. Population III was seeded in July and the fruits examined were autumn-grown. Certain of these fruits provided respiration records that form a striking contrast with corresponding summer-grown fruit. The implication of the respiration studies is that the vast majority of autumn-winter-grown fruits when isolated at appropriate stages of maturity and kept individually at 12.5° C. are in a different physiological state from the summer-grown fruit. The difference is interesting from the point of view of storage because it involves a great extension of life at the temperature employed. The plants of Population IV were started in winter, but fruited in spring and represent the spring population.

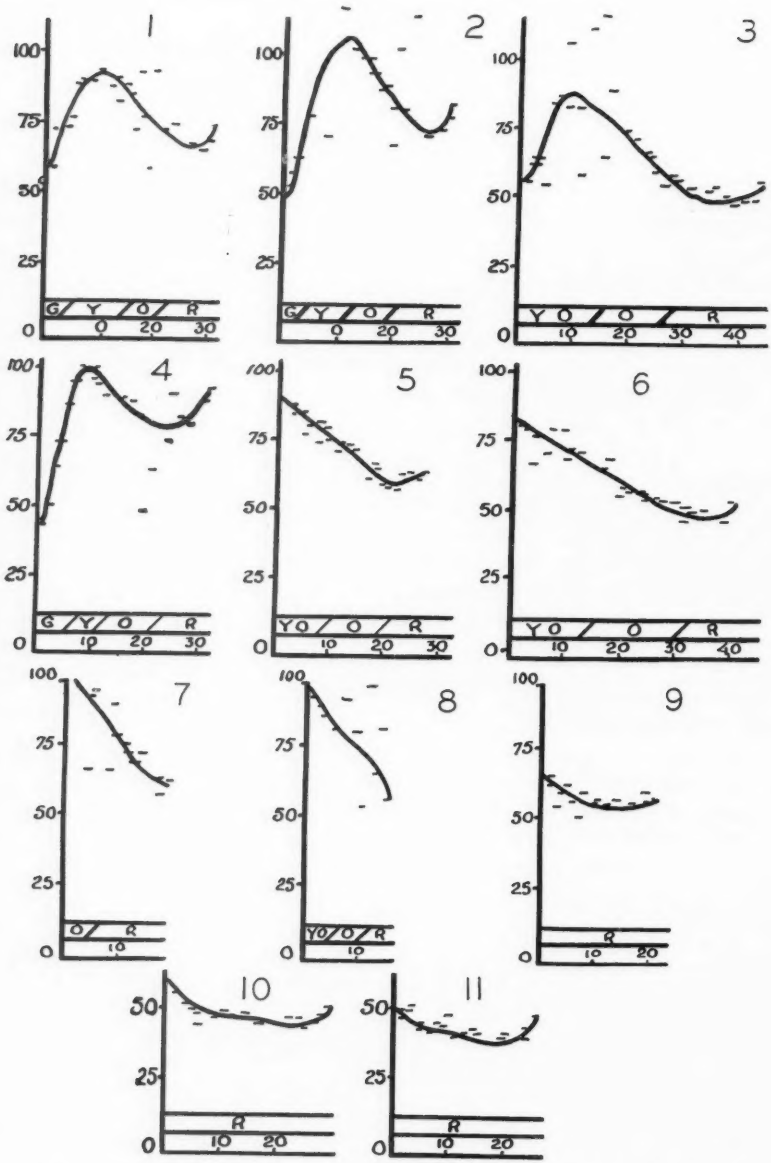
Experimental Results

Fruits of Populations I and II

The respiration records of eleven fruits from Population I are given in graphic form in Figs. 1 to 11. Before turning to the data, comment must be made on certain fluctuations of great amplitude occurring in some of the earliest records obtained. These fluctuations are quite distinct from the normal fluctuations found in every respiration record. Their origin was traced to stoppage of the air stream by deposition of barium carbonate in the narrow delivery tubes with which the Pettenkofer tubes were originally furnished. Replacement of the delivery tubes removed the difficulty. Points known to be affected by this circumstance are neglected in drawing the lines.

Records 1 and 2 are of fruit classified as Stage III, mature-green at the time of picking. Respiration rises steadily to a maximum of 100 to 110 cc. CO_2 in 12 days and then drops to about 70 cc., completing the rise and fall in about 27 days. After a few days of steady rate tissue breakdown occurs, carbon dioxide output rises, and the determinations end.

The ripening color changes commence almost immediately after the beginning of the record, so we must conclude that the fruits were in the last stages of mature greenness at the time of picking. The fruits change from yellow-green to orange during the rise of respiration and the transition to red is



FIGS. 1–11. *The respiratory drift in storage (at 12.5° C.) of fruits of Population I. Vertical axis indicates cc. CO_2 per 10 kg. per hr.; horizontal axis, time in days and color changes in the fruit (G = green, Y = yellowing YO = yellow-orange, O = orange, R = red).*

accompanied by falling respiration rates. The correlation of the visible ripening color changes with a rise and fall of respiration confirms Gustafson (4), who found that ripening tomatoes pass through these respiratory phases. This phenomenon appears to be an almost general property of senescent plant tissues. The "senescent rise of respiration" was long ago observed by Blackman (2) in starving leaves, and more recently by Blackman and Parija (3), Kidd and West (5, 6, 7) and others, in isolated fruits such as apples and pears. It must be regarded as the conventional mode of behavior of ripening fruits, departures from which should be noteworthy.

The ready identification of the form of these records with the conventional senescent phases of respiration makes it clear that the records are devoid of initial phases which would indicate a change of physiological state as an immediate result of isolation or cooling. Hence the initial rates of the records may be taken as approximations to rates characteristic of the fruit before detachment from the plant. A series of such initial rates obtained for fruits isolated at various states of maturity ought, therefore, to indicate the manner in which the respiration is changing whilst the fruit is on the plant. This in turn should enable us to determine the relation between the course of respiration on the plant and the course after isolation at the storage temperature, as well as any changes which the seasonal factor may induce in this relation. The initial rates of these two fruits are of the order of 50–60 cc. CO_2 per kg.-hr.

Records 3 and 4 are of fruits which were picked just as the ripening color changes began, and at picking were classified as in Stage IV of maturity, very slightly in advance of Fruits 1 and 2. The respiration records are correspondingly similar and clearly represent the conventional senescent respiratory phases accompanying, in the same manner as before, the visible changes of color. The initial rates are also of the same order as those of Records 1 and 2.

The next pair of records (5 and 6) are of fruits picked in the yellow-orange or fifth stage of maturity. These records are quite different from the four that have just been examined. The initial rates are of the order of 90 cc. CO_2, which is approximately that of the peak values of Records 1 to 4, and the form of the records is that of a simple, gentle decline. In Fruit 6 breakdown occurred two weeks later than in Fruit 5. The change in color from yellow-orange, to orange, to red, accompanies these falling rates just as it accompanies the declining respiration phase in Records 1 to 4.

Judging from the initial rates of Records 5 and 6, respiration on the plant has increased substantially between Stages IV and V. We conclude, therefore, that Fruits 5 and 6 had already passed into the senescent rise of respiration before they were picked, that they were picked near to the peak of the rise and that their records of respiration after picking represent the declining arms of senescent phases which remain to be completed, on a modified time scale, at the low temperature.

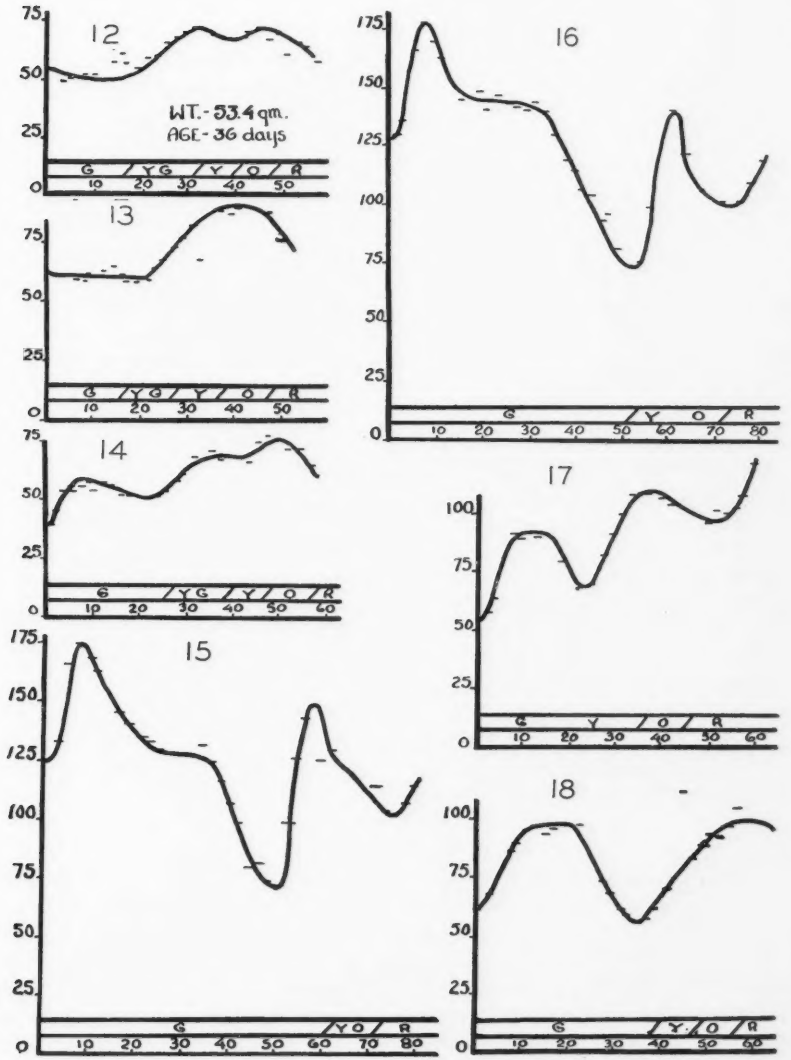
Stage VI fruit is represented by Numbers 7 and 8. The form of these records is again a decline, but this time the decline is steep. The initial rates of about 100 cc. CO_2 per hr. are very high, indicating probably a further increase over Fruits 5 and 6 of respiration while still on the plant. The conclusion suggested is that Fruits 7 and 8 were picked at or just after the maximum of the senescent rise on the plant and they then completed what remained of the decline after isolation at 12.5° C.

Records 9, 10 and 11 are of fruits which were full red at picking. If the indications of the other records have been properly interpreted, the larger part of the senescent rise and fall of respiration in these fruits should have been passed through on the plant and we should, therefore, expect that the terminal portions only would remain to be gone through in isolation. The form and pitch of Records 9, 10 and 11 obviously fulfil this expectation.

Seven growing-green fruits of Population II were examined, and their respiration records are numbers 12 to 18. The first three were picked at the end of the growing-green stage; the remaining individuals were much less advanced in that stage when they were removed from the plant.

Records 12 to 14 begin with a more or less steady or slightly declining phase, introduced in Record 14 by an initial rise. The steady phase continues with more or less random fluctuation for 20 to 25 days, after which the rate of respiration goes up. This increase in rate is correlated in each record with the external ripening color changes in the fruit, hence we must conclude that the rise and subsequent decline represent the senescent rise of respiration. These records differ in form from Records 1 to 4 (mature-green fruit) essentially in the presence of the extended steady phase antecedent to the senescent rise. The presence of the steady phase suggests that the fruit passes through a similar phase while on the plant. If so, then Fruits 12 to 14 must have been picked well before this phase was past, so that they continued in it for 20 days after isolation before passing into the senescent phase. Fruits 1 and 2, on the other hand, must have been picked after this phase was over, for they are in the senescent rise from the beginning of the record. Moreover, the initial rates of Fruits 1 and 2 are of the same order as those of 12, 13 and 14, *i.e.*, 50 to 60 cc. CO_2, so that whatever interval of development on the plant separates the two groups of fruits, it must be an interval of relatively steady respiration.

If Fruits 12, 13 and 14 were picked just as they reached the border line between late growing- and mature-green, Fruits 15 and 16 were certainly remote from the border line and were in fact the youngest fruits to have been examined in any of the populations. Fruits 17 and 18, though assigned with Numbers 15 and 16 to the physiological Class I (early growing-green) were considerably farther advanced than the latter. They were taken from the field to supplement the fruits of Population II because at the time of the experiment, the greenhouse crop did not afford examples of fruit at precisely the right stage.



FIGS. 12–18. *The respiratory drift in storage (at 12.5° C.) of fruits of Population II. Vertical axis indicates cc. CO_2 per 10 kg. per hr.; horizontal axis, time in days and color changes in the fruit (G = green, Y = yellowing, YO = yellow-orange, O = orange, R = red).*

Dealing first with the physiologically younger pair of fruits, Numbers 15 and 16, inspection of the respiration record reveals an extremely complex form, yet one which is evidently characteristic of the state at the time of isolation, since the two records correspond in every detail. The records start off with initial values of about 130 cc. CO_2. and for the first week respiration rises until a maximum of about 180 cc. CO_2 is reached. Then a fall sets in, which is interrupted by a temporary steady state lasting for about 10 days, and then resumed until a transitional minimum of about 70 cc. is reached after 50 days. The minimum gives way at once to a sharp rise which is correlated in time with the first visible ripening color changes and is, therefore, to be identified as the senescent rise. This soon reaches a maximum and the records terminate in the manner of Records 1 to 4.

For our present purpose it is not necessary to enter more fully into the characters of these records and their physiological significance. They seem to be special cases of the general form which also underlies the respiration records of starving leaves and immature apples. In conformity with this, the initial rates of 130 cc. CO_2 are very high, indicating an altogether higher order of respiratory rate in the young growing fruit on the plant than at any subsequent stage of development.

Records 17 and 18 begin with an initial rise similar to that found in Records 15 and 16 and also in Record 14. Since this cannot be the senescent rise, it must have some other significance. Experience shows that this initial effect is characteristic of fruits isolated in the early stages of development when starch is abundant. As starch diminishes, this feature disappears from the records. Amongst the present data it is found in the records of all the very starchy fruits, in one out of three of the fruits which are rapidly losing their starch, and in no others. When the initial effect is over, respiration is found to be in a steady state similar to that which interrupts the declining phase in Records 15 and 16. After the steady phase the second limb of the declining phase appears, followed by a transitional minimum and the senescent rise. The form of Records 17 and 18 is therefore that of Records 15 and 16 with the first half of the declining phase omitted. The initial rates are of a very much lower order, suggesting that the interval separating the stage represented by Fruits 15 and 16 from that represented by Fruits 17 and 18 is characterized by a very rapid fall of respiration in the fruit on the plant.

We may now consider the whole series of summer-grown fruits. Fig. 36 is a somewhat schematized plotting of the initial rates of the isolated fruits against time. This graph represents approximately the sequence of changes in respiration rate which characterizes the growth and development of the summer-grown fruit on the plant. When we compare the series of respiration records given by isolated fruits with this graph and with each other, it is clear that the isolated fruit continues in modified form at the storage temperature the sequence begun on the plant, and does not recapitulate phases that have already been passed through. The records of fruits picked in later stages of maturity may therefore be regarded as derived from those of fruits

picked in earlier stages, by progressive omission of the early phases of the record. The metabolic history at the storage temperature is therefore determined by the point reached in the normal sequence at the time of picking.

The normal sequence of the tomato fruit growing and maturing on the plant is of the same form as that made familiar by extensive investigations on the apple and pear, and the physiological changes underlying it are presumably of general significance. The rule that the isolated fruit continues the sequence (in modified form) seems also to apply to other fruits, so that the summer-grown tomato gives no evidence of unconventionality in these respects. It is, therefore, interesting to find that autumn-winter-grown tomatoes, isolated in certain stages of development, appear to depart from this rule in a manner and to an extent that seems to have significance certainly for the scientific and possibly also for the practical aspects of the problem of preserving the fruit in the living state.

Fruits of Population III

The anomalous behavior of fruit from this as well as from subsequent autumn-winter populations of plants draws attention to the seasonal differences in growth conditions with which this variation appears to be correlated. The components of the seasonal factor for which some records are available are temperature and illumination.

The records of temperature in the greenhouse are incomplete. But fortunately it is not difficult to chart the course of mean temperature during the missing portions of the records from experience with sufficient certainty for the present purposes. In Fig. 37 the weekly mean maxima and minima are given separately for day and night. With the temperature chart is another which shows the whole growth period of each population and the interval of time during which the fruits were sampled. A similar set of charts for weekly mean hours of light and weekly mean hours of sunlight in relation to growth and sampling is given in Fig. 38. From these figures it is possible to form some conception of the manner in which these components of the seasonal factor varied from one population to another.

It is especially clear in connection with illumination that the autumn-winter population was grown under conditions that were radically different from those obtaining during the growth and sampling of the others. The summer populations were grown when this factor was rising from the minimum, and the fruit was sampled when it was maximal. The winter-spring population was grown when illumination was at first minimal, then rising, but the fruit was sampled when illumination was about half maximal. Population III on the other hand, was grown when illumination was falling from the maximum and the fruit was sampled at the minimum of this factor. There can be little doubt that in respect of light periodicity, intensity and quality, the autumn-winter period differs sharply from the summer periods, while the spring period is intermediate, but appears to have more affinity with the summer than with the autumn.

Mean temperatures tend to be steady and moderate from October to April, but reach high values in the other months of the year. In respect of temperature the autumn and winter-spring periods differ chiefly in the higher temperatures prevailing during the early growth of the autumn plants. The larger part of the two growth periods and both sampling periods were characterized by steady moderate temperature. The larger part of the growth period of the summer populations and their sampling periods were characterized by high maximum temperatures.

As a whole, the temperature and illumination components of the seasonal factor indicate a sharp difference between the summer and the autumn-winter periods, with the winter-spring period partaking of the characters of both. It might be supposed, therefore, that any influence season might have upon the physiological state of the fruit would be chiefly manifested in differences between fruits of summer and autumn-winter populations, with winter-spring fruits in an intermediate position. Owing to the fact that fruits of Stages III and IV are not represented in the winter-spring sample of the first year's work, it cannot be decided from the present body of data alone whether fruits of the autumn-winter type are found amongst the winter-spring population. But subsequent experiments show that the winter type does extend into the early spring. The transition from the winter to the summer type appears to occur in March to April.

We have little doubt that the physiological composition of the fruits of the several populations is a correlative of season. But the effect of the seasonal factor may be to influence the distribution of types which are actually present in all seasons rather than to evoke in one season a type quite unknown in another. Consequently to avoid confusing this issue until more is known we shall refer to the summer and winter types merely as the "conventional" and "anomalous" types respectively.

TABLE III

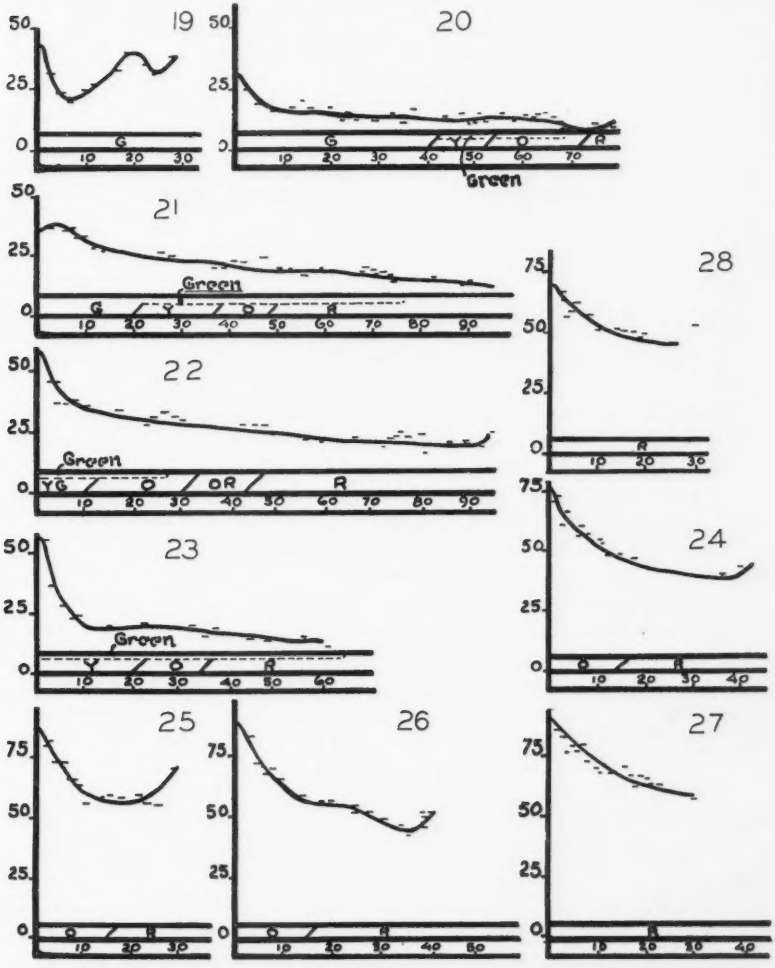
THE RELATION BETWEEN AGE AND PHYSIOLOGICAL STATE IN POPULATION III

Fruit No.	20	21	22	23	24	25	26	27	28
Stage	III	III	IV	IV	VI	VI	VI	VII	VII
Age	47	54	62	53	52	52	54	52	54
Growth interval	Oct. 3 Nov. 19	Sept. 26 Nov. 19	Sept. 28 Nov. 19	Oct. 9 Dec. 1	Sept. 28 Nov. 19	Oct. 10 Dec. 1	Sept. 26 Nov. 19	Sept. 28 Nov. 19	Sept. 26 Nov. 19

Table III gives for each fruit of Population III the stage of maturity at which it was picked, its chronological age and growth interval. The growth intervals vary only from 47 to 62 days and all but two lie between 52 and 54 days, yet the maturity stages represented lie between mature-green (III) and full red (VII). This sort of physiological heterogeneity amongst fruits of the same chronological age is found in other populations also but not to

the same extent as in this population. It will be shown later that the heterogeneity within a single fruit is also more marked in the fruit of this than in fruit of the other populations.

The respiration records are given in Figs. 19–28. The first of these, Number 19, is of a fruit which was classified at picking as on the border of growing-green and mature-green. A premature tissue breakdown associated with rising respiration developed in this fruit. Nothing of the sort occurred



FIGS. 19–28. *The respiratory drift in storage (at 12.5° C.) of fruits of Population III. Vertical axis indicates cc. CO_2 per 10 kg. per hr.; horizontal axis, time in days and color changes in the fruit (G = green, Y = yellowing, YO = yellow-orange, O = orange, R = red).*

in any other fruit of this population. The breakdown did not appear to have an infective origin, but there is no critical evidence that this is so. It might be characteristic of an extreme variant in a physiologically heterogeneous group. It is best, therefore, to reserve comment on this individual.

Fruits 20 and 21 were picked in the mature-green stage. The respiration records are low in pitch and the initial rates are of the order of 35 to 40 cc. CO_2. Record 21 gives slight indication of an initial rise such as characterized the earlier stages of corresponding fruits in Population II. From the initial value the rate drops more or less gently to a prolonged phase of low, almost steady, slightly declining respiration. This phase is of remarkable duration. The experiments had to be terminated before it came to an end and before the fruits showed the slightest sign of breakdown. The records, though very long, are nevertheless incomplete. In fruit of later populations records of this type have been observed which went on for 130 days.

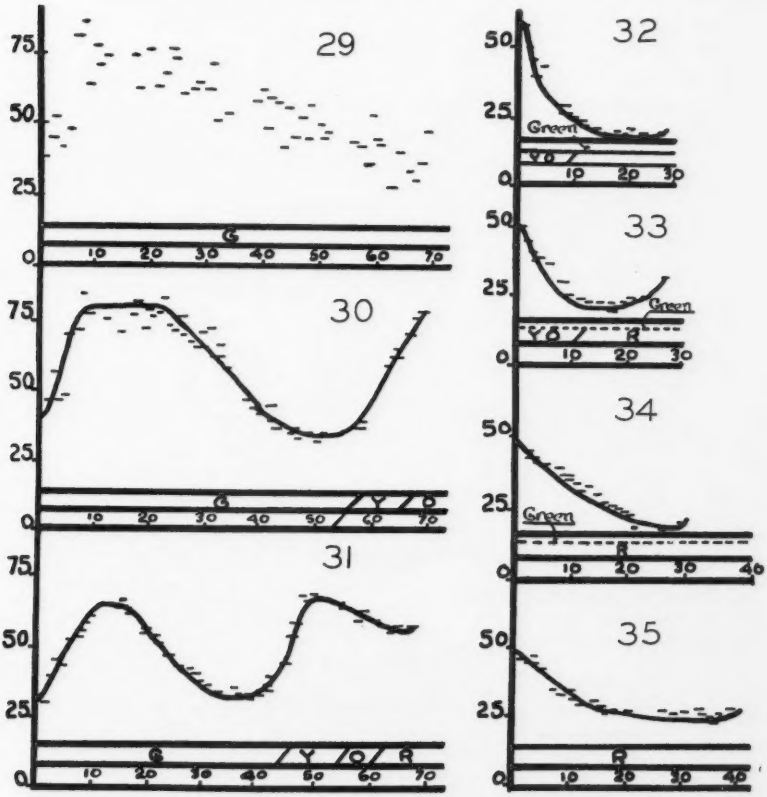
The only other fruits to pass through a long steady phase of anything like this appearance were Numbers 12, 13 and 14 of Population II. But in the latter, the steady phase passed into the senescent rise when the external color changes began, whereas in the present fruits the steady phase continues quite unbroken throughout the period of color change and long past the point at which the fruits had become full, ripe red. The only unusual feature of the ripening color changes was a marked tendency of an island of tissue in the immediate vicinity of the calyx to lag behind the rest of the fruit. Ultimately ripening was complete. The persistence of the calyx end indicates, however, that these fruits are characterized by a higher order of cell heterogeneity than summer-grown fruits.

It is evident that the steady phases of Records 12, 13 and 14 cannot be homologized with those of Records 20 and 21. Indeed the present records have little in common with those of summer-grown fruit picked at any stage whatever. They represent the anomalous type, the characteristics of which are a decline sometimes prefaced by an "initial effect" passing into a phase of steady respiration greatly extended in time, during the unbroken course of which the ripening color changes occur. The essence of the anomaly is the absence of the senescent rise, a phenomenon occurring regularly in relation to ripening not only in our summer-grown fruit but also observed as the rule for tomatoes by Gustafson (4). This type of record appears to indicate a state of physiological stability quite unusual for tomatoes and is associated with a duration of life at 12.5° C. several times as great as that characterizing corresponding fruits of the conventional type.

The next two fruits (Records 22 and 23) were isolated at Stage IV, just as they were commencing to turn yellow. The records are of the same general form as Numbers 20 and 21. The initial rates of 57 and 60 cc. CO_2 are higher than those of Records 20 and 21, from which it appears that while yet on the plant the fruits actually pass into the usual rising phase of respiration as the ripening color changes supervene. In this they resemble the conventional type. But the remarkable thing is that the yellowing fruits of the present

population, after they are picked, do not proceed with the senescent rise in the manner of the conventional type (Records 1 to 4). On the contrary, the records decline from the beginning, passing into an extended phase of precisely the character of that in Records 20 and 21. The ripening color changes also occur in the absence of a senescent rise, and the extraordinary duration of life which accompanied the stable state of Numbers 20 and 21 occurs in the present pair as well.

This series of records does not happen to include examples of fruits isolated in Stage V. Records 24 to 28 are of fruits picked in the orange (VI) and red (VII) stages of maturity. The first three were orange when picked. In the fruit of the summer populations this color, whether developed on the plant or after picking, at 12.5° C. is associated with the peak values of the senescent rise of respiration. This appears to be true of the attached autumn-winter



FIGS. 29–35. *The respiratory drift in storage (at 12.5° C.) of Population IV. Vertical axis indicates cc. CO_2 per 10 kg. per hr.; horizontal axis, time in days and color changes in the fruit (G = green, Y = yellowing, YO = yellow-orange, O = orange, R = red).*

fruits also for we find the initial rates of Records 24 to 26 in the vicinity of 80 to 90 cc. CO_2. The form of the records is that of fruits of corresponding maturity in the summer populations and, as before, evidently represents the declining arm of the senescent phases. No stable state is established and in 25 to 40 days breakdown occurs.

Of the two fruits picked full red, Number 27 has a high initial rate and a record very much the same in form as those of fruits that were orange when picked. Presumably it was actually on the border line of orange and red. The record of the other ripe fruit has a lower initial rate and in all its characteristics is indistinguishable from the corresponding ripe fruits of Population I. (Records 9 and 10). It would seem, therefore, that the stable characteristics which distinguish the isolated unripe fruits of autumn-winter populations from the corresponding summer-grown fruits are not manifested in the autumn-winter fruit if it is permitted to pass a certain point of maturity before it is picked. The evidence is that this point is roughly marked by the external appearance of red pigmentation in the fruit.

Fruits of Population IV

This is the winter-spring population and the records of respiration of the fruits studied are given in Figs. 29–35. The first of these is characterized by an unusual scatter of points referable to error in the measurement of the low

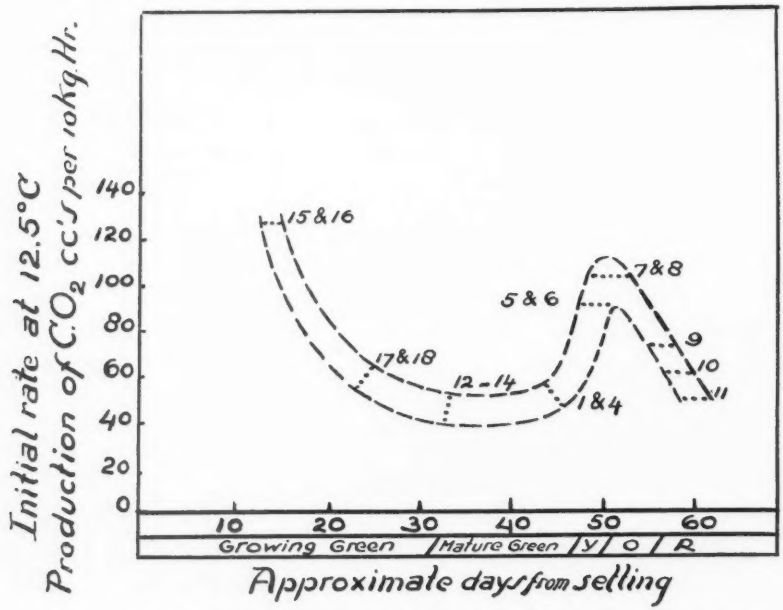


FIG. 36. *The drift of respiration of tomato fruits of Populations I and II on the plant as determined from the initial rates of the stored fruits of the summer-grown populations.*

CO_2 output of the very small fruit. There is evidently an "initial effect" such as appears in the records of all very young fruits, and thereafter a decline, but all details of form are obscured. The two fellow records (30 and 31) are of fruits not so early in the growing-green stage and their form is evidently that of Records 17 and 18, Population II, but they are of lower pitch. We conclude that winter-spring fruit isolated in March at the growing-green stage is physiologically comparable to corresponding summer fruit.

The next stages of maturity were not represented in fruits of this population, but the examination of subsequent winter-spring fruits indicates that the anomalous type appears, provided the fruits are picked early in the spring as well as at the appropriate stage of maturity. Later, the conventional type predominates.

Fruits 32 and 33 were picked just as the red pigment began to appear superficially. Their relatively high initial rates indicate that the senescent rise had set in while they were still on the plant. The records conform to the conventional type and are comparable to Records 5 and 6 of Population I except again for their lower pitch. Similarly Records 34 and 35 of fruits picked full red are comparable to Records 9, 10 and 11 of Population I.

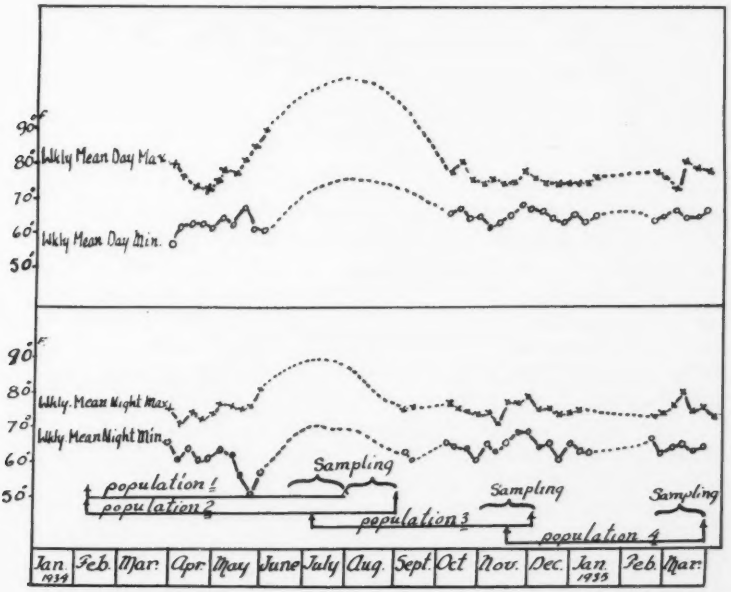


FIG. 37. *Temperature records in the greenhouse during the growth of Populations I to IV, showing the weekly mean day maximum, the weekly mean day minimum, the weekly mean night maximum and the weekly mean night minimum. These temperature records are not complete but are connected by dotted lines which indicate the probable temperatures approximating those obtained from outside temperatures. The period of growth and the sampling period for each population are indicated at the bottom of the figure.*

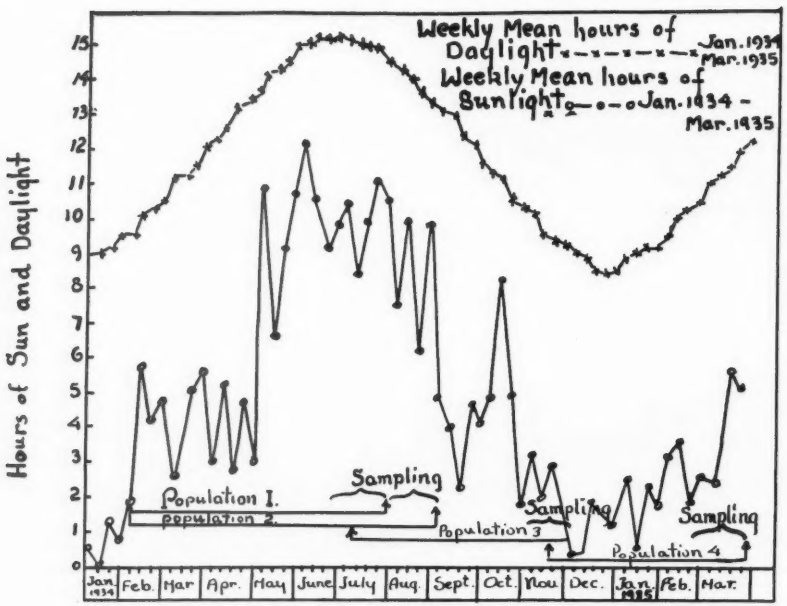


FIG. 38. *The weekly mean hours of daylight and the weekly mean hours of sunlight, together with the growing period and the sampling period for each of the four populations.*

The evidence provided by the data examined in detail in this paper, as well as of those for subsequent populations of tomatoes, indicates that in the course of ripening while on the plant, fruits pass through the senescent rise of respiration irrespective of the time of year in which they are grown in the greenhouse. During the late spring and summer the ripening processes of isolated fruit at 12.5° C. are also accompanied by the senescent rise of respiration in the conventional manner and are characterized by the lack of durability normal to this fruit. At other times of the year the course of metabolism in the fruit isolated at 12.5° C. depends upon the stage of maturity at which it is picked. If ripeness is approaching, as indicated by the external appearance of red pigment, the course of metabolism conforms to the conventional type and the fruit is not durable. But the autumn-winter-early-spring fruit, if picked approximately mature-green, departs from the conventional type. The respiration record reveals an extended period of remarkably steady rate while ripening is in progress and for long afterwards, suggesting a metabolically stable condition quite different from that found in fruit of the conventional type. This anomalous type of ripening is associated with a very much greater durability of the fruit both at 12.5° C. and afterwards, if removed to a higher temperature. The quality of such fruit in respect of texture and flavor when ripe is quite satisfactory.

Acknowledgments

The author is indebted to Mr. W. Hugh Smith, at whose suggestion this work was begun and to Dr. J. H. L. Truscott for guidance during the greater part of the time it was in progress. Professor G. H. Duff of the University of Toronto made many valuable suggestions in the formulation of the data and in criticism of the manuscript. To those gentlemen the author offers his thanks.

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The "BLACK-POINT" OR "KERNEL SMUDGE" DISEASE OF CEREALS[1]

By J. E. Machacek[2] and F. J. Greaney[3]

Abstract

A seed disease of wheat, rye, and barley, characterized by a more or less conspicuous brown or black discoloration of the kernel, particularly in the region of the embryo, frequently reduces the seed value and usually the sales value of affected grain. A comprehensive review of the relevant literature is given. The name "kernel smudge" is suggested for this disease. Two species of *Alternaria* (*A. tenuis* and *A. Peglioni*) and two of *Helminthosporium* (*H. sativum* and *H. teres*) are the fungi chiefly associated with it in Manitoba. The kernel smudge caused by *Alternaria* cannot be accurately distinguished from that caused by *Helminthosporium*, without a laboratory examination of the seed.

Extensive trials with wheat have demonstrated that the *Alternaria* type of kernel smudge does not affect to any marked extent seed germination, plant emergence, intensity of root rot, and yield in the subsequent crop. It was established, however, that *H. sativum* does reduce germination, seedling emergence, and the yield, and at the same time causes an increased amount of root rot in this crop.

Under Manitoba conditions infection of the kernels arises from air-borne spores which are usually deposited in the largest numbers at about the time the kernels are maturing. Contrary to some observations made elsewhere, the disease does not result in shrunken kernels; the largest kernels are frequently infected, and the small, shrunken ones usually free from the disease. Apparently the reason for this is that the large kernels force open their covering glumes, thus affording access to air-borne spores, whereas the glumes of small kernels remain closed and exclude such spores.

The seed value of grain attacked by the virulent *(H. sativum)* type of kernel smudge was increased considerably when such grain was dusted with suitable organic mercury dusts (ethyl mercury phosphate or methyl mercury nitrate). Dusting with copper carbonate was relatively ineffective. The development of kernel smudge in the maturing crop was not prevented by dusting the growing plants with sulphur.

Introduction

A disease of cereals, frequently referred to as "black-point", occurs in most grain-growing areas of the world and causes considerable loss. The literature relating to it is fragmentary and not infrequently conflicting: the exact nature and cause of the disease are not clearly defined. In this paper a general review of the results obtained by other workers is given, and an attempt is made to discuss different aspects of this disease in the light of observations and experiments made in Manitoba during the eight-year period, 1929 to 1936.

Economic Importance

This disease has been reported as present in Canada, the United States of America, Argentina, Germany, Italy, Morocco, South Africa, India, and Java, and probably it occurs wherever cereals are grown. It attacks wheat, rye, and barley, as well as certain cultivated and wild grasses. As yet, however, few investigators have attempted to estimate the loss resulting from

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this pathological condition of the seed. Bolley (5), in referring to the damage caused by *Helminthosporium sativum* to wheat grown in North Dakota during 1920, stated that this organism was very abundant and particularly destructive "in destroying stools and in the production of black points in the grain". According to Weniger (52), black-point of wheat caused a loss of 0.5% in North Dakota in 1925, but it was less severe that year than usual.

An idea of the prevalence of this disease in Manitoba can be gained from Table I in which is given the percentage of discolored kernels found in eight varieties belonging to the wheat groups *Triticum durum* and *T. vulgare*. These varieties were grown at Winnipeg during the seven years, 1929 to 1935. It would appear from this table that the *durum* wheat varieties are more susceptible to this disease than are the *vulgare* varieties. This relation was most noticeable in 1935.

TABLE I

THE PREVALENCE OF DISCOLORED KERNELS IN THE THRESHED GRAIN OF WHEAT VARIETIES GROWN AT WINNIPEG, MAN., DURING THE 7-YEAR PERIOD, 1929–1935.
(PERCENTAGE OF KERNELS DISCOLORED IN COUNTS OF 2,000)

Variety	1929	1930	1931	1932	1933	1934	1935	Mean
T. durum								
Acme	1.3	22.2	11.0	42.8	30.8	69.0	65.3	34.6
Pentad	0.8	—	21.7	37.3	40.7	63.7	93.6	42.9
Mindum	2.6	11.5	15.7	—	—	24.2	55.0	21.8
T. vulgare								
Marquis	0.4	4.1	2.6	—	0.7	—	10.8	3.7
Reward	0.0	—	0.2	—	0.7	—	4.0	1.2
Ceres	—	—	0.0	—	1.8	—	5.3	3.5
Pentad × Marquis (R.L. 723)	—	5.8	27.6	2.4	—	—	—	11.9
Pentad × Marquis (R.L. 726)	—	—	18.3	2.3	0.7	5.4	—	6.7

The presence of discolored kernels in the threshed grain lowers the market value of a crop. To command the highest price, grain, whether wheat for milling or barley for malting, should be uniformly bright in color. Grain merchants usually associate poor color with poor quality of the grain. Millers of durum wheats have a particular reason for avoiding grain containing a large proportion of discolored kernels. They find it exceedingly difficult to remove the dark specks which appear in the semolina milled from such grain, and when these specks appear in the finished products, macaroni, vermicelli, etc., the quality of the product is appreciably reduced. Furthermore, discoloration of the seed indicates a diseased condition and often the presence of parasitic fungi that may attack and destroy the seedling plants if seed thus affected is sown.

Nomenclature

"Black-point" was a term first used in 1913 by Bolley (5) to describe a wheat-kernel discoloration supposedly due to fungal invasion. Previous to this date, however, Zöbl (53) in 1892, and Puchner (35) in 1897, used the

adjectives "braunspitzige" and "schwarzspitzige", respectively, to describe analogous symptoms in barley. Ravn (36) followed the nomenclature of Zöbl. Coons (11), in 1918, used the term "black point" for a disease of wheat in which the main symptom was a blackening of the kernel tips. He found a bacterium, tentatively identified as *Bacterium viridi-lividum*, in the discolored tissues. Louise Stakman (45) isolated a fungus which she considered to be *Helminthosporium sativum* P., K., & B. from discolored kernels of wheat and rye. She stated that the disease symptoms produced by this fungus on the kernel differed distinctly from those of the so-called "black tip". The "black tip" disease referred to here is presumably that caused by the basal glume-rot organism, *Bacterium atrofaciens* McCulloch. Weniger (51) and Evans (15) found that *H. sativum* was usually associated with a kernel-discoloring disease of wheat which they called "black point"

Drechsler (14) showed that while both *H. sativum* and certain unidentified species of *Alternaria* were associated with the blackening of wheat kernels, the disease symptoms produced by these two types of organisms were slightly different. He retained the nomenclature used by Weniger and Evans. Rosella (37) and Miège (29) designated the kernel-blackening disease as "Moucheture". Peyronel (34) reported that in Italy the disease was known as "Puntatura". Pasinetti (33), describing a disease of San Martin wheat in which the kernels became blackened as the result of some unknown physiological derangement within the plant, stated that the name "Punta Nera" was applied to this disease by the Argentine growers. Dastur (12) showed that *H. sativum* was the principal cause of "black point" in wheat grown in India. Simmonds (41) applied the name "smudge" to wheat-kernel discolorations caused by *H. sativum*. Mead (27) used the same name for a similar disease in barley. In a more recent paper, Christensen and Stakman (10) refer to barley-kernel discolorations caused by *H. sativum* and species of *Alternaria* and *Fusarium* as "seed blight".

For many years bacterial and fungal discolorations of wheat, barley, and rye have been commonly termed "black point". In recent years, however, different workers, by observation and experiment, have established that definite and important differences exist between these two types of seed discolorations. The term "black-tip", referred to by Louise Stakman (45), aptly describes the bacterial type of kernel discoloration, while the term "smudge", as employed by Simmonds (41), more adequately describes the symptoms produced by fungal infection. The term "smudge" does not, however, indicate the location of the disease on the host plant. It is suggested, therefore, that the name "kernel smudge" be applied to the disease of wheat, rye, and barley, in which a dark or pale-brown diffuse discoloration occurs on any part of the kernel, particularly in the region of the embryo, and which is produced by fungi, especially those belonging to the arbitrary group Moniliales–Dematiaceae of the Fungi Imperfecti. Wheat kernels attacked by "kernel smudge" are illustrated in Plate I, F and G.

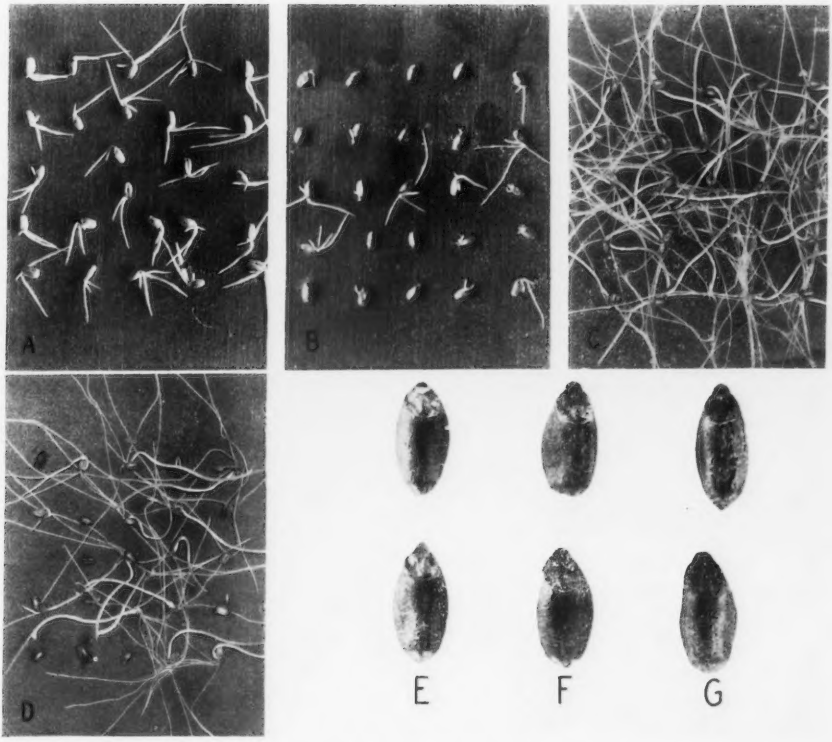

PLATE I. *A-D. The effect on germination and growth in Pentad wheat of two types of kernel smudge. A and C, kernel smudge (Alternaria type) of 1934 crop. B and D, kernel smudge (Helminthosporium sativum type) of 1935 crop. A and B, growth after 3 days. C and D, growth after 6 days. E-G. Kernel smudge on kernels of Pentad wheat. E, healthy kernels. F, discolored kernels (Alternaria type) of 1934 crop. G, discolored kernels (Helminthosporium sativum type) of 1935 crop.*

Etiology

Zöbl (53) isolated several different fungi from discolored barley kernels, but concluded that one of these fungi, *Cladosporium herbarum*, caused the discolorations. Peyronel (34), however, showed that while *C. herbarum* was sometimes associated with this disease it was not the cause. Ravn (36) found that *Helminthosporium teres* occurred in discolored barley kernels. Bolley (5) concluded that species of *Alternaria* and *Helminthosporium* were the chief causes of seed discolorations in wheat, although he found species of *Fusarium* in the diseased kernels as well. Hoffer (21) isolated *Fusarium* sp. and *Macrosporium* sp. from discolored wheat kernels. Palm (32) reported that, in Java, wheat kernels sometimes were infected by *H. gramineum*. According to Drechsler (14) the fungus isolated by Palm was not *H. gramineum* but *H. sativum*. Louise Stakman (45) isolated *H. sativum* from the discolored kernels of wheat and rye. Elsewhere (46) she reported that *Alternaria*, *Botrytis*, *Fusarium*, and *Tilachlidium* were found associated with the seeds of cereals. The strains of *Alternaria* which she isolated belonged to two general types. Evans (15) reported that 77.6% of the kernels in a sample of *durum* wheat were infected by *H. sativum*. Bassi (2) identified the causal pathogen of a seed disease of wheat as *H. gramineum*. This fungus, reported by Bassi as causing a conspicuous discoloration of the kernels, is believed by the writers to have been misnamed, being not *H. gramineum* but *H. teres*. The latter has been frequently isolated from discolored wheat kernels by the writers.

Christensen (8) found *H. sativum* in discolored seed of wheat, rye, and several grasses. Weniger (51) considered that *H. sativum* was the principal cause of kernel discoloration in wheat. Henry (19, 20) isolated a number of unrelated fungi from discolored wheat kernels, but *H. sativum* and a species of *Alternaria* were found most often. Drechsler (14) concluded that both *H. sativum* and *Alternaria* spp. caused seed discolorations in cereals. Rosella (37) found two species of *Alternaria*, *A. tenuis* and *A. Peglioni*, causing seed discolorations of wheat grown in Morocco, and (38) that *H. gramineum* caused a similar disease in barley. O'Gara (31) isolated *Podosporiella verticillata* from diseased wheat kernels. Dastur (12) showed that *H. sativum* was responsible for most of the discolorations of wheat seed produced in India. Waldron's (49) work suggested that wheat kernels were probably infected with *H. sativum* as frequently as with species of *Alternaria*. Simmonds (41) showed that *H. sativum* was a very common contaminant of seed wheat. Christensen and Stakman (10) isolated a number of different fungi from diseased barley kernels, but found that species of *Helminthosporium*, *Fusarium*, and *Alternaria* were present most often. Isolation studies made by Machacek (23), in 1925, indicated that *Alternaria* spp. were the fungi most commonly present in the kernels of wheat and rye grown in Saskatchewan, while *H. sativum* and species of *Fusarium* occurred much less frequently in these cereals.

During the five-year period, 1931 to 1935, the writers investigated the fungus flora in discolored kernels of wheat, barley, and rye. In the first four

years of this period the predominating fungi in such kernels were *Alternaria tenuis* Nees and *A. Peglioni* Curzi; the number of isolates of these two fungi exceeded by three times the total of the other fungi isolated. *Helminthosporium sativum* and *Fusarium* spp. were found only seldom. In 1935, however, most of the discolored wheat kernels in the samples examined were found to be infected with *H. sativum*, while *Alternaria* spp. were recovered much less frequently than in the previous years of the investigations. Occasionally, particularly in barley, exceptions were found. For instance, in 1934, a sample of discolored barley seed yielded several fungi in the following proportion: *H. teres*, 73.0%; *H. sativum*, 12.0%; *Alternaria* spp., 11.2%; *Fusarium* spp., 3.8%. In this sample, *H. teres* apparently caused most of the seed discoloration.

Methods of Isolation

A number of different techniques have been used by various workers to isolate fungi from discolored kernels. The most important difference in these techniques was in the method used to surface-sterilize the seed. Hoffer (21) sterilized wheat seed by dipping it in an alcoholic solution of mercuric bichloride. Norton and Chen (30) pre-soaked the seed for 10 to 12 hours in water and then sterilized it according to the method of Hoffer. Henry (20) did not pre-soak the seed, but first disinfected it with an alcoholic solution of mercuric bichloride, and then rinsed it with alcohol to remove the superfluous metallic salt adhering to the seed.

Although chemical treatments have been used extensively for disinfecting the seed of cereal crops, considerable criticism concerning their use for surface sterilization of infected kernels has been made. Louise Stakman (45) objected to the use of mercuric bichloride for seed which was infected with both *Helminthosporium sativum* and *Alternaria*. She found that *H. sativum* was more easily destroyed by this chemical than was *Alternaria*. Thus it would be presumed that, by this method of sterilization, *Alternaria* would be isolated more frequently than *H. sativum* from diseased seed, even when possibly more of the kernels were infected with *H. sativum* than with *Alternaria*. Mead (28) isolated from infected kernels, sterilized with silver nitrate, fungi with pale mycelium more frequently than fungi with dark mycelium, and thereby confirmed the finding just referred to, that fungi may respond differently to a single chemical.

To avoid any such selective action by chemicals in seed disinfection, attempts have been made to develop better methods for surface-sterilizing seed. Simmonds (41) removed surface-borne contaminants from seed by washing it in sterile water. In some cases he used a surface-tension-reducing substance in order to wet the seed thoroughly. He (42) constructed a device to wash seed or other plant parts. He was able to isolate a greater variety of fungi when he washed the diseased seed than when he disinfected it with chemical reagents. Atanasoff and Johnson (1) subjected barley seed infected with *H. sativum* to dry heat (95°–100° C. for 30 hr.) and found that this fungus was either inactivated or destroyed by the treatment, for the seedlings

arising from the treated seed were perfectly healthy. Mead (28), having investigated several methods of destroying or removing surface contaminants from the seed of cereals, concluded that the use of chemicals was satisfactory where completely sterile seed was desired, but that a more accurate determination of the fungus flora of seed could be obtained by the "Washer" method of Simmonds (42). He found that the use of dry or wet heat was unsatisfactory for purposes of seed sterilization.

As it is sometimes desirable to know the value of discolored grain for seed purposes, a practical and efficient method of diagnosing the type of seed discoloration is required. In an attempt to devise a satisfactory method whereby the seed value of large samples of grain could be determined, Simmonds and Mead (43) subjected a large number of samples to the following tests: (i) visual examination, (ii) centrifuge tests for the determination of surface-borne fungi, (iii) germination tests in sterile sand. In addition, the fungi within the seed were determined by the usual plating-out method, and, to some extent at least, by a microscopical examination of the kernel pericarp. The results indicated that the methods employed gave a very complete determination of the fungi within and on diseased kernels.

In the course of the present investigations, a simple, practical, and efficient method was used to determine the presence of fungi in seed affected by kernel smudge. This method is a modification of that used in the "plating-out" of diseased seed, the chief difference being that the surface-sterilized kernels are plated in soft agar, instead of on the surface of hardened agar, in Petri dishes. The kernels are first sterilized by being soaked for three minutes in ethyl alcohol-mercuric bichloride solution (one part 95% alcohol to three parts 1 : 1000 solution mercuric bichloride). While the seed is being disinfected, melted potato-dextrose agar is poured into sterile Petri dishes, and cooled to a temperature of about 60° C. The sterilized kernels are then planted in the soft agar, after which the agar is cooled as quickly as possible. Thus, the soft agar partially envelops the kernels, and the excess alcohol and mercuric bichloride on the surface of the kernels are absorbed or inactivated by the agar. In this process the seed is handled only once after undergoing sterilization, and hence the opportunity for it to become recontaminated by air-borne spores is greatly reduced. After a few days of incubation, the fungi within the diseased kernels emerge and grow on the surface of the agar, from where they can be readily isolated for further study.

The effectiveness of this method of isolating fungi from discolored seed was demonstrated during an examination of two samples of Pentad wheat, one from the 1934 and the other from the 1935 crop. A macroscopical examination of the seed showed that both samples were severely attacked by kernel smudge, there being little difference between the samples either in degree of discoloration or in the number of discolored kernels. Germination tests on moist blotting paper, however, indicated that there was a very marked difference in the ability of the two samples to germinate (Plate I, A-D). The 1934 seed germinated well, and although fungus mycelium emerged from the

TABLE II

RELATIVE FREQUENCY WITH WHICH *Helminthosporium sativum*, *Alternaria* SPP., AND *Fusarium* SPP., WERE ISOLATED FROM DISCOLORED KERNELS OF PENTAD WHEAT PRODUCED IN MANITOBA IN 1934 AND 1935

Crop year	Percentage of discolored kernels yielding		
	H. sativum	*Alternaria* spp.	*Fusarium* spp.
1934	1.9	81.4	0.00
1935	85.3	14.0	0.02

kernels, the seedling remained healthy for the entire experimental period of six days. On the other hand, the 1935 seed germinated very poorly, and many of the seedlings were badly diseased. By the modified plating-out method described above, 81.4% of the discolored kernels in the 1934 sample yielded *Alternaria*, while this fungus was isolated from only 14.0% of the 1935 seed. On the other hand, 85.3% of the discolored kernels in the 1935 seed yielded *H. sativum* as compared with 1.9% in the 1934 seed (Table II).

Further tests were made with these two samples of Pentad wheat. After surface-sterilization, seed of each sample was sown in pots of autoclaved soil, and grown under controlled conditions in the greenhouse. The plants were lifted and examined at the end of 10 days. The results of this test, given in Table III, show that the 1934 seed germinated 100% and produced but a small percentage of diseased seedlings, while of the 1935 seed 78.0% of the kernels germinated and 89.7% of the seedlings were diseased. Thus, the

TABLE III

THE EFFECT OF KERNEL SMUDGE ON GERMINATION, AND ON THE INCIDENCE OF DISEASE IN PENTAD WHEAT GROWN IN STERILE SOIL UNDER GREENHOUSE CONDITIONS

Crop year	Dominant fungus in seed	Percentage of seeds germinated	Percentage of plants diseased	Root-rot disease rating
1934	*Alternaria* spp.	100.0	15.0	6.2
1935	*H. sativum*	78.0	89.7	52.4

results of the germination and greenhouse tests confirmed the results obtained by the modified plating-out method, and demonstrated that, although the 1934 and 1935 samples of Pentad wheat were apparently equally affected by kernel smudge, only the 1934 seed was suitable for seed purposes. These results were substantiated by field tests with 1934 and 1935 seed of Pentad wheat at Winnipeg in 1936 (Table X).

Pathogenicity of Fungi Isolated from Discolored Seed

Several workers have attempted to establish the pathogenicity of fungi isolated from discolored kernels of wheat and other cereals. In this earlier work various methods of inoculating plants with these fungi were employed. Louise Stakman (45) inserted the spores of a *Helminthosporium*, presumably *H. sativum*, between the glumes of Marquis wheat, and covered the infected

parts of the head with moist cotton and oiled paper caps for 48 hr. The mature kernels of heads thus inoculated were badly discolored. Evans (15) applied a water suspension of *H. sativum* spores to the heads of Acme and another wheat (unnamed), and enclosed the inoculated heads in glassine bags for one or two days. Many discolored kernels developed. Christensen (8) obtained many discolored kernels in cereal and grass varieties when he sprayed the growing plants with a water suspension of *H. sativum* spores. He found that the reactions of these hosts to other manifestations (leaf blotch and root rot) of the disease caused by this fungus were not correlated with the amount of disease developed in the seed. Henry (20) obtained diseased kernels when he placed dry spores or mycelium of *H. sativum* on the heads of wheat. He sprayed the heads with sterile water before and after inoculation. Scott and Sallans (40) found that wheat kernels would become discolored if a water suspension of *H. sativum* was sprayed on the heads, or injected between the glumes of the florets. Sallans (39), using similar techniques, induced infection of wheat kernels by a species of *Alternaria*. Mead (27), by employing any one of the following methods, namely, spraying the heads with, or immersing them in, a water suspension of spores of *H. sativum*, or placing, by means of a pipette, drops of the spore suspension between the spikelets and rachides, was able to induce kernel infection in barley with each of the following fungi: *H. sativum*, *Gibberella Saubinetii*, *Fusarium* sp., and *Alternaria* sp. Peterson* produced kernel smudge by injecting a spore suspension of either *H. sativum* or *Alternaria tenuis* into the florets of wheat. Each fungus was subsequently recovered from the discolored kernels of the inoculated plants.

In the present studies, kernel smudge has been produced in five varieties of wheat by the employment of any one of the following methods: (i) dusting spores of *H. sativum* on the glumes of growing plants, (ii) spraying the wheat spikes with a water suspension of spores of this fungus, (iii) injecting, by means of a hypodermic needle, the spore suspension into the leaf sheath enclosing the spike of plants in the "boot" stage of growth. Plants inoculated by either of the first two methods were incubated, immediately after inoculation, in moist chambers for two days; and then placed on a greenhouse bench and grown to maturity. The inoculated plants in all cases produced a large number of discolored kernels, from which *H. sativum* was recovered.

Pathological Histology

Bolley (3) was one of the first to observe fungus hyphae within the integuments, and around the embryo, of discolored kernels of wheat. Later (5), in referring to *Alternaria* sp. particularly, he showed that this fungus first penetrated the integuments, spreading afterwards in the spaces surrounding the embryo. He noticed, furthermore, that the scutellum often became diseased, in which case the food supply contained in the endosperm of the kernel was made unavailable to the germinating embryo. Owing to the lack

* *Unpublished data of Dr. R. F. Peterson, Dominion Rust Research Laboratory, Winnipeg, Manitoba, Canada.*

of food supply the young seedling was frequently unable to break through the integuments, and, when it did, it was weak and easily destroyed by pathogenic fungi such as are common in the soil. Weniger (51) found that the mycelium of *Helminthosporium sativum* was similarly distributed in the discolored kernels of *durum* wheat, and that during germination the plumule and rootlets became diseased prior to their emergence from the seed.

Peyronel (34) showed that the first symptom of disease in a kernel was a discoloration of the lodicules, organs in close proximity to the scutellum. He found at the base of these lodicules a tissue of thin-walled cells, rich in nutrients. After fertilization of the flower occurred, this tissue withered and became a very suitable substratum for the growth of fungi, the mycelium of which often penetrated the dead cells and then invaded the integuments over the embryo. Peyronel believed that the rapidity of desiccation of the lodicular tissues was a determining factor in the development of seed discolorations, a factor which he thought would affect the amount of disease in different seasons. He found also that the discoloration of the integuments was due to the formation of a brown pigment, and, although the pigment was confined chiefly to the layer of horizontal cells overlying the epicarp, it sometimes occurred in the epicarp tissue as well. The cells of the aleurone layer were also often discolored, but not those of the embryo. Dastur (12) found that, in wheat kernels infected with *H. sativum*, the fungus hyphae invaded the kernel integuments but did not reach the embryo. This freedom of the embryo from infection, he concluded, explained why many discolored kernels germinated normally.

Christensen (8) found that the mycelium of *H. sativum* within the seed of wheat remained viable for at least six and one-half years. In the present study it has been demonstrated that the mycelium of *H. sativum* and *Alternaria* spp. grew well after a dormancy of four years within the kernels. The evidence indicates that these fungi can maintain themselves in a dormant condition for several years within infected seed of cereals.

Factors Influencing the Development of Kernel Smudge

The prevalence of kernel smudge in a cereal crop is probably determined by the combined action of a number of factors. In the present investigations three of these factors, namely, air-borne inoculum, stage of host maturity, and varietal resistance, were given especial consideration.

Air-borne Inoculum

Stakman *et al.* (47), in their studies on the spore content of the upper air, observed that spores of many fungi, including *Alternaria* and *Helminthosporium*, were often lifted to considerable heights and thus could be dispersed by air currents over wide areas. Little definite information is available in the literature concerning the seasonal variation of air-borne inoculum of these fungi and the relation of such inoculum to the prevalence and development of kernel smudge. During the course of the present investigation, an attempt

was made to gather information concerning the relative number of spores of *Alternaria* and *H. sativum* in the air over Manitoba.

Each year, from 1932 to 1936, during the summer months, vaselined microscope slides were exposed daily in spore traps located at three widely separated stations in Manitoba (Winnipeg, Morden, and Brandon). The spores of *Alternaria* and *H. sativum* intercepted by one square inch of slide surface during each exposure period of 24 hr. were counted. These counts were used to compute the averages for intervals of one week each during the growing season. There is much of interest and value in these spore-counting data, but a detailed discussion of the results would occupy too much space, therefore, only the results of the spore counts made at Winnipeg during these years are presented (Table IV, Fig. 1).

The data in Table IV show that during June, July, and August, of each year, spores of *Alternaria* were numerous in the atmosphere. An appreciable increase in the number of these spores occurred during late July and early

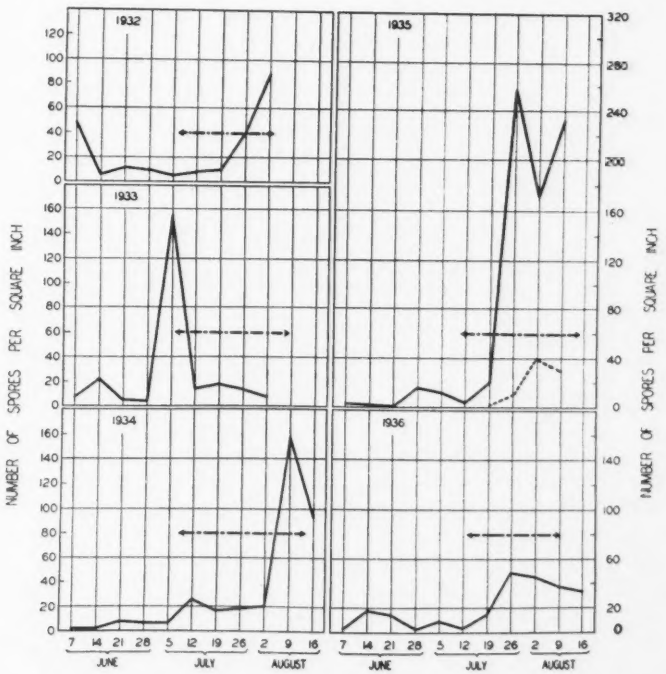

FIG. 1. *The seasonal variation in number of air-borne spores of kernel-smudge fungi, as represented by weekly averages of daily spore counts in June, July, and August, of each of the 5 years, 1932-1936. Alternaria spp. represented by a continuous line, Helminthosporium sativum (1935) by a broken line. The interval between date of heading and harvest each year is indicated by a broken, arrowhead line.*

August in 1932, 1934, 1935, and 1936. Sallans (39) made counts of airborne spores of *Alternaria* during June, July, and August in 1928 and 1929, at Indian Head and Saskatoon, Saskatchewan, and found a similar sharp increase in the number of spores during late July. This increase was particularly noticeable in 1928 at Indian Head.

While spores of *Alternaria* were intercepted in large numbers on the slides during each year of this investigation, spores of *H. sativum* were usually intercepted only in very small numbers. In Fig. 1, therefore, graphs showing the occurrence of this fungus on the slides in 1932, 1933, 1934, and 1936, are omitted. In 1935, however, there was a very decided increase in the number of *H. sativum* spores intercepted by the slides at three stations, and, therefore a graph showing the occurrence of this fungus in 1935 is given.

TABLE IV

THE SEASONAL VARIATION IN THE NUMBER OF SPORES OF KERNEL SMUDGE FUNGI CAUGHT ON GLASS SLIDES AT WINNIPEG, MAN., DURING THE FIVE YEARS 1932–1936. (WEEKLY AVERAGES OF DAILY COUNTS OF NUMBER OF SPORES ON ONE SQUARE INCH OF SLIDE DURING JUNE, JULY, AND AUGUST)

Week ending	1932		1933		1934		1935		1936	
	Alternaria	*H. sativum*	*Alternaria*	*H. sativum*	*Alternaria*	*H. sativum*	*Alternaria*	*H. sativum*	*Alternaria*	*H. sativum*
June 7	48.2	0.0	7.0	0.0	2.0	0.0	2.9	0.2	2.1	0.1
14	5.1	0.0	22.0	0.3	2.0	0.0	1.4	0.0	18.1	2.4
21	11.4	0.0	4.0	0.6	8.0	0.0	0.7	0.0	14.3	0.1
28	9.6	0.0	3.1	0.0	7.0	0.0	15.6	1.0	2.4	0.3
July 5	3.7	0.0	156.0	0.0	7.0	0.0	12.9	0.9	9.0	0.7
12	8.3	0.0	15.5	0.0	26.3	0.0	3.0	0.0	2.4	0.3
19	12.9	0.0	18.1	0.0	18.4	0.0	19.9	1.8	15.0	0.4
26	40.3	0.0	13.2	0.0	19.1	0.0	257.9	11.2	47.1	0.8
Aug. 2	88.3	1.7	8.2	0.0	19.5	0.0	170.9	40.8	45.7	11.5
9	—	—	—	—	149.3	0.0	229.7	35.7	38.5	2.0
16	—	—	—	—	93.2	0.0	—	—	35.2	2.0

An examination of the data on daily spore counts of *Alternaria* and *H. sativum*, presented graphically in Fig. 1, shows that, in each year (except at Winnipeg in 1933), the largest numbers of spores of these fungi were present in the air over Manitoba during the period of wheat growth from heading to maturity. This period of growth for Marquis wheat is indicated in each graph in Fig. 1 by a broken, arrow-head line. At Winnipeg, in 1933, a very marked increase in the number of *Alternaria* spores occurred during the last week of June and the first week of July, before the plants had reached the heading stage. The cause of this exceptionally early increase is not clearly understood.

From daily counts of *Alternaria* spores, it was evident that the atmospheric spore load varied greatly from day to day. An examination of the meteorological data for the summer months showed that there was a close correlation

between wind direction and the number of *Alternaria* spores on the slides. The greatest numbers were caught after a southerly wind had blown for one or two days. A change of wind direction invariably resulted in an appreciable decrease in the number of spores. It was concluded, therefore, that the greater part of the spores of *Alternaria* originated in areas to the south of Manitoba, and that these air-borne spores were widely disseminated by winds over the grain-growing areas of this province.

Although the spore counts are an index of the relative number of spores in the air, they are not necessarily a reliable index of the number of spores that are deposited on living hosts in any given area. The spore traps were not designed to measure the number of spores deposited at ground level, or even to indicate the number of spores in the atmosphere at the level of grain crops. Thus, the data presented in Table IV are only of value in so far as they give a general indication of the prevalence of fungus spores in the air from day to day. Nevertheless, the value of such information in predicting destructive outbreaks of plant diseases has been demonstrated. The occurrence of a severe outbreak of kernel smudge (*H. sativum* type) in Manitoba was predicted in 1935 from the information gained in a study of the spore content of the air. As indicated earlier, this type of kernel smudge was epidemic in that year.

The number of *Alternaria* spores present in the air during the summer of 1935 greatly exceeded the number of *H. sativum* spores and consequently it was expected that a large proportion of the discolored kernels would be infected by *Alternaria*. Isolation studies (Table II) did not confirm this expectation, because the majority of the diseased kernels yielded *H. sativum*.

Why the *H. sativum* type of kernel smudge predominated in 1935 is not altogether clear. From the work of Bolley, Dastur, Peyronel, and Weniger, it appears that infection of wheat kernels by *H. sativum* and *Alternaria* proceeds in a somewhat similar manner. If air-borne inoculum of both fungi is present, as in 1935, it would be expected that many of the kernels would probably be infected by both fungi. On this assumption both fungi should be isolated from such kernels, for the technique of isolation used by the writers does not favor the isolation of one more than the other. It would appear, therefore, that some factor, probably an antagonism between the two fungi in the seed, may have been responsible for the high percentage of *H. sativum* isolates from the 1935 seed.

There is some evidence in support of this hypothesis. It has been the experience of the present writers that, when *Alternaria* spp. and *H. sativum* are cultured together, the *Alternaria* cultures are not compatible with those of *H. sativum*, that is, the association hinders the growth of *Alternaria*. Recently Johnson* found that when colonies of *Alternaria* sp. and *H. sativum* were cultured together in the same Petri dish the adverse influence of *H. sativum* on *Alternaria* increased with a decrease in the distance separating

* *Unpublished data of Dr. T. Johnson, Dominion Rust Research Laboratory, Winnipeg, Manitoba.*

the two inocula on the agar. When the inocula were placed side by side the growth of *Alternaria* was very limited while that of *H. sativum* was not hindered in the least.

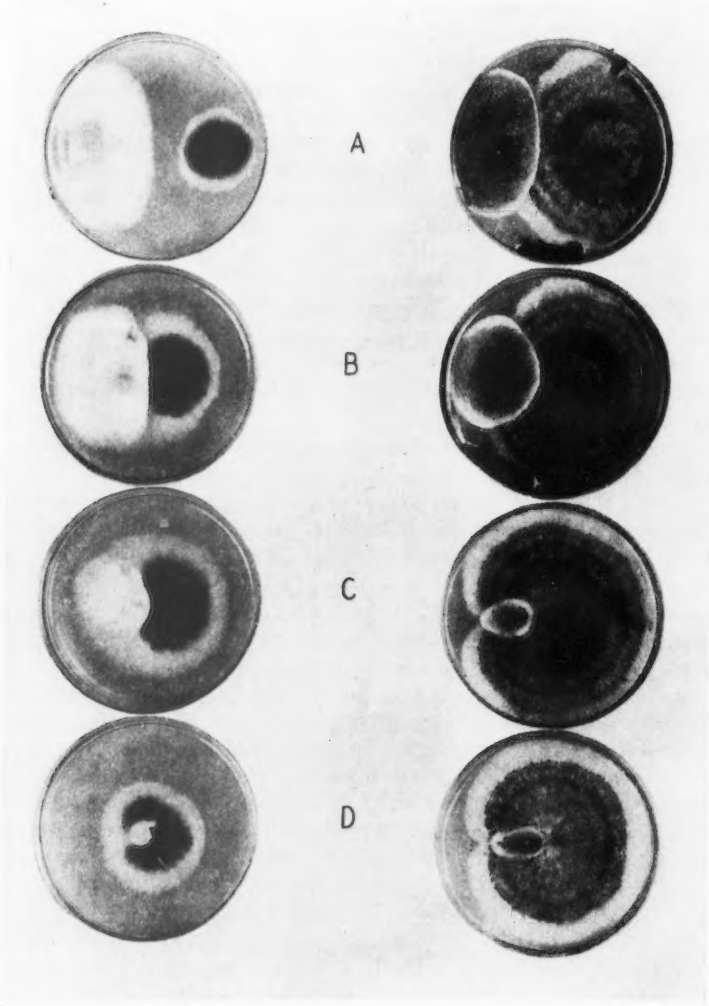

FIG. 2. *The inhibiting action of Helminthosporium sativum on Alternaria tenuis. Growth of colonies on Czapek's agar (left) and potato-dextrose agar (right) after 8 days. A. Inocula placed 6 cm. apart. B. Inocula placed 2.2 cm. apart. C. Inocula placed 1 cm. apart. D. Inocula placed side by side on surface of agar.*

The experiments of Johnson have been repeated, and his results confirmed, by the present workers. Two fungi, *Alternaria tenuis* and *H. sativum*, isolated from wheat kernels affected by kernel smudge, were cultured on potato-dextrose agar and Czapek's agar. The inoculums of the two fungi were placed at four different distances apart. Cultures grown for a period of one week at room temperature appeared as shown in Fig. 2. Repetition of the test produced similar results.

On the basis of the results obtained by Johnson and the writers, it appears possible that, in the absence of *H. sativum*, infection of the kernels by *Alternaria* occurs in the normal way, but when infection by both fungi is more or less simultaneous the repressive action of *H. sativum* on *Alternaria* prevents the complete establishment of the latter fungus in the kernel, while the other grows more or less normally. It is possible, too, that a kernel already infected by *Alternaria* may be invaded, partly at least, by *H. sativum*. Furthermore, it may be assumed that, when attempts are made to isolate these fungi from diseased kernels, the outward growth of *Alternaria* is inhibited, or at least retarded by the antagonistic action of *H. sativum*. On this assumption, it could be expected that the number of isolates of *H. sativum* from the 1935 crop of wheat, previously referred to, would greatly exceed those of *Alternaria*, even though the latter fungus had the greater chance to infect the kernels. The results of 1935 (Table II) substantiate this expectation, for there was only slight evidence of *Alternaria* infection in the 1935 seed. Although the above hypothesis is based on analogy, it seems to be a reasonable explanation of the problem encountered in 1935. The fact that *H. sativum* inhibited *Alternaria tenuis* in a similar manner, whether potato-dextrose agar or Czapek's agar was used, adds further weight to the conviction that the hypothesis is at least partially correct, as the chances that the same phenomenon would occur in the kernels of cereals are greatly increased.

Stage of Host Maturity

Kernel smudge is essentially a disease of the seed. Apparently, in nature, the interval during which infection of the seed may take place is restricted to the period of kernel development. Christensen (9) stated that *H. sativum* can infect the wheat kernel during any stage of its growth. Peyronel (34) found that the fungi causing kernel discoloration infected the growing kernels within a few days after the flowers had been fertilized. Scott and Sallans (40) showed that most of the infection occurred during the blossoming period of the flowers, but also that infection could occur at any later stage of kernel growth. Sallans (39) found that the characteristic discoloration appeared most often when the kernels were inoculated during or after the "soft dough" stage of growth. Rosella (38) observed that *H. sativum* invaded barley spikes while they were still enclosed by the leaf sheaths. The fungus penetrated the sheaths, reached the young inflorescence, passed through the glumes and rachides, and finally invaded the kernels. Henry (20) showed in his experiments that *H. sativum* invaded wheat kernels chiefly during the "milk" stage of their growth.

The actual time during which the kernels become infested under field conditions is still a matter of conjecture. In the case of the *H. sativum* type of kernel smudge, the kernels are invaded either indirectly, as the work of Rosella (38) and the present studies have shown, or directly, as a number of workers (8, 14, 27, 40, 45) have demonstrated. The species of *Alternaria* causing kernel smudge do not appear to be so actively parasitic as *H. sativum*, for they do not ordinarily attack the glumes and rachides until these have withered and died. Therefore, it is reasonable to assume that under ordinary conditions the infection of the kernel by *Alternaria* spp. is more or less direct, that is, the fungus initially comes in contact with some part of the growing kernel. The kernel may become infected either when the florets are expanded during the blossoming period, or when, at or near plant maturity, the increase in kernel size separates the glumes.

There are two reasons for assuming that, under Manitoba conditions, infection of the kernel takes place during the more mature stages of plant growth. First, there is very little available inoculum at the time of the first opening of the florets (Fig. 1 and Table IV), and second, it has been found that the infected kernels are usually plump and well developed (Table V). From the literature on kernel discolorations in cereals, however, it appears that infection of the kernel by *H. sativum* leads to reduction in size of the kernel. On the other hand, the writers, working under greenhouse conditions, found that it was only when they inoculated very immature kernels with *H. sativum* that the mature kernels were shrunken. During the years of the present investigation, shrunken kernels with discolorations typical of kernel smudge were seldom found, but the disease was commonly observed on large kernels.

Further evidence, that under Manitoba conditions kernel smudge occurs from late infection of the kernels, can be obtained by examining the relation between stem rust of wheat and the prevalence of kernel smudge. It is a well known fact that severe attacks by stem rust result in an appreciable reduction in kernel size. In the present investigation the shrunken grain from a rusted crop was examined to determine the prevalence of discolored kernels. Very few shrunken kernels were found to be discolored. It seems that, owing to the reduced size of the kernels, the enveloping glumes did not separate widely as the kernels matured, and hence an avenue for the kernel smudge fungi was not provided. The negative correlation found by Goulden (17) between the amount of kernel smudge in a wheat variety and its susceptibility to stem rust may be explained on this basis.

Varietal Resistance

Bolley (5) showed that, in the development of seed discolorations, the variety of host is an important factor: the hard wheats were generally more susceptible than the soft wheats. Dastur (12) found that wheat varieties differed in their susceptibility to the *H. sativum* type of this disease. Christensen (8) stated that the reactions of different wheat varieties to *H. sativum*,

as defined by the occurrence of leaf lesions, were not correlated with the percentages of discolored kernels in the grain. Other workers (15, 52) have shown that the *durum* wheats are more susceptible to seed discoloration than are wheats of the *vulgare* type, a finding which has been confirmed by the present studies (Table I).

In 1931, Peterson* found a wide range of susceptibility to kernel smudge among wheat varieties. In general, varieties of the durum type were more susceptible to the disease than were varieties of the *vulgare* type. In a series of pure lines of a *durum* × *vulgare* (Pentad × Marquis) wheat cross, the susceptibility of the *durum* parent was carried over to most of these lines, although a few of them showed a high resistance to the disease. In other wheat crosses (H-44-24 × Reward, and Double Cross × Ceres), tested in 1931, a similar result was obtained. The experiments were repeated in 1932 with results very similar to those of 1931. In 1933, he made greenhouse tests to determine the reaction of wheat varieties to kernel smudge. These tests showed that the greenhouse reactions of certain wheat varieties to *H. sativum* and *Alternaria* sp. were closely correlated with the field reaction of these varieties.

Goulden (17) made several interesting observations on the relation of host variety to the amount of kernel smudge. His results, based on an analysis of the relation between several pairs of variables in 52 pure lines of a H-44-24 × Marquis cross, demonstrated that there was a high degree of correlation between certain pairs of these variables. These results established that the percentage of kernel smudge in the crop was significantly, but negatively, correlated with (i) infection by Form 21 of wheat stem rust (*Puccinia graminis tritici* Erikss. and Henn.), (ii) seed color, and (iii) length of time required to mature the crop. There was a slight, but significant, positive correlation between yield and the percentage of kernel smudge.

Effect of Kernel Smudge on Size and Weight of Kernels

Peyronel (34), Rosella (37), and Waldron (49) showed that discolored wheat kernels are frequently larger and heavier than are sound kernels. Tests were made to determine whether a similar situation prevailed under Manitoba conditions. The seed of Pentad wheat, produced in sulphur-dusted and undusted plots at Winnipeg during 1933, 1934, and 1935, was used in these tests. From the threshed grain of both dusted and control plots of each year, three samples were taken at random. For each of these samples the weight per 1000 kernels of each type of seed (healthy and smudged) was determined. The average 1000-kernel weight for each group of three samples in these tests is given in Table V.

The data in Table V confirm the findings of previous workers (34, 37, 49), and show that, in Manitoba, kernel smudge is usually very prevalent in fully developed kernels of wheat. In each year of the test the discolored kernels were heavier than the healthy kernels, although the difference in weight

* *See reference, page 91.*

TABLE V

RELATIVE WEIGHT OF 1000 KERNELS OF HEALTHY AND DISCOLORED SEED FROM SULPHUR-DUSTED AND UNDUSTED PLANTS OF PENTAD WHEAT 1933, 1934, AND 1935. (AVERAGE WEIGHT IN GRAMS OF THREE 1000-KERNEL SAMPLES)

Crop year	Plants dusted with sulphur			Plants not dusted		
	Healthy seed	Diseased seed	Increase in weight of diseased seed	Healthy seed	Diseased seed	Increase in weight of diseased seed
1933	26.9	27.8	0.9	27.0	28.0	1.0
1934	29.7	33.9	4.2	29.1	32.6	3.5
1935	16.5	20.2	3.7	15.3	18.5	3.2
Mean	24.4	27.3	2.9	23.8	26.4	2.6

varied from year to year. Dusting growing plants with sulphur resulted in a slight increase in kernel weight, but it did not disturb materially the relation between the weight of healthy and diseased kernels.

Influence of Kernel Smudge on Plant Growth, Incidence of Seedling Blight and Root Rot, and on Yield

EXPERIMENTAL METHODS

Field experiments to determine the effect on the subsequent crop of planting seed affected by kernel smudge were made in 1932, 1933, and 1934. The experiments were designed to study several aspects of the disease. The system of plot replication used consisted of six randomized blocks of at least eight plots each. Within each block there was complete randomization of varieties and kinds of seeds (diseased and healthy). The individual plots consisted of two rod rows placed one foot apart. In one of these rows a predetermined quantity (15 gm. of wheat of the *vulgare* type and 18 gm. of the *durum* type) was sown, while one hundred kernels of the same variety and same kind of seed were sown, spaced about two and one-half inches apart, in the other row. The former row furnished data relative to yield and the amount of kernel smudge in the threshed grain of the plot, while the latter provided information concerning the number of plants and the degree of infection on individual plants.

Notes on the number of plants and amount of root rot were obtained from ten to fifteen days before harvest. The plants in the 100-seed row of each plot were pulled, counted, and examined individually, and the severity of root-rot infection recorded. The degree of root rot in each plot was subsequently determined by the formula (modified) of McKinney (25), and the experimental data analyzed by the procedure described by Fisher (16). To estimate the odds of significance, however, Snedecor's (44) F value, the direct ratio of the variances, was used. The complete experiment was made at Winnipeg in 1932, and repeated in 1933 and 1934.

The Effect of Kernel Smudge on Seed Germination and Plant Emergence

Puchner (35) found that discolored kernels of barley germinated poorly, and, after germination, often produced seedlings with disease lesions on the leaves. Bolley (3, 4, 5) stated that wheat seedlings arising from diseased kernels were usually weak and, when grown under field conditions, subject to disease. Güssow (18) found that discolored wheat seed germinated as well as did healthy seed, but that the seedlings resulting from the discolored seed were not very thrifty. Louise Stakman (45, 46), Christensen (8), Christensen and Stakman (10), Weniger (51), Drechsler (14), Henry (20), Simmonds (41), and Scott and Sallans (40), found that wheat kernels attacked by *H. sativum* germinated poorly, and that, in most cases, diseased seedlings resulted from such seed. Dastur (12) showed that the value of a crop was appreciably reduced when seed infected with *H. sativum* was planted. He observed that the discolored seed germinated poorly, and that the seedlings from such seed usually became blighted. He found, also, that the amount of injury to the seedlings depended to a considerable extent on the time of seeding and on the weather conditions prevailing at the time of seed germination. Rosella (37) found that discolored wheat and barley kernels germinated better in sand than on nutrient agar in Petri dishes. Miège (29) obtained no differences in germination when healthy and discolored wheat seed was planted in field soil. The results of a three-year test made by Stening (48) showed that discolored wheat seed germinated about 5.5% less than did healthy seed. Waldron (50) obtained only slight reductions in germination when discolored wheat seed was planted. Machacek (23) found that discolored kernels selected from a number of wheat varieties germinated poorly, and observed that such seed, when surface-sterilized and plated-out on nutrient agar or planted in sterile soil, gave rise to seedlings the coleoptiles and rootlets of which were diseased.

One of the objects of the field experiments made at Winnipeg in 1932, 1933, and 1934, was to determine the effect of sowing discolored seed on plant emergence in wheat. Several varieties of wheat were planted. The results for the three years are given in Table VI. They show that, although the differences in plant emergence observed between healthy and diseased seed were not significant in any given year of the test, there was a general tendency for the discolored seed to give lower emergence than the healthy seed. However, as the discolored seed used in 1932, 1933, and 1934, was mostly infected with *Alternaria* sp., it was not to be expected that there would be any marked difference in the germinability of healthy and discolored seed planted in these years. If the seed had been infected by *H. sativum*, however, it is probable that a significant difference in germination of the healthy and diseased seed would have been obtained, as the results in Table III lead one to expect, and those in Table X substantiate.

TABLE VI

EFFECT OF PLANTING HEALTHY AND DISEASED (KERNEL SMUDGE) SEED OF WHEAT IN FIELD PLOTS ON PLANT EMERGENCE IN 1932, 1933, AND 1934

Analyses of Variance

Year	Source of variance	Degrees of freedom	Sum of squares	Mean square	F	5% point
1932	Replicates	5	1415.35	283.07		
	Varieties	3	179.73	59.91	—	—
	Error (a)	15	945.90	63.06		
	Total	23	2540.98			
	Seed (healthy and diseased)	1	143.52	143.52	3.55	4.35
	Seed × varieties	3	49.06	16.35	—	—
	Error (b)	20	808.92	40.44		
	Total	24	1001.50			
1933	Controlled error	8	1485.24	185.65		
	Varieties	5	2880.24	576.04	9.91	2.66
	Error (a)	22	1278.68	58.12		
	Total	35	5644.16			
	Seed (healthy and diseased)	1	153.12	153.12	2.57	4.17
	Seed × varieties	5	1069.46	213.89	3.58	2.53
	Error (b)	30	1790.92	59.69		
	Total	36	3013.50			
1934	Controlled error	8	1660.91	207.61		
	Varieties	3	368.23	106.08	1.20	3.49
	Error (a)	12	1064.34	88.69		
	Total	23	3043.48			
	Seed (healthy and diseased)	1	11.02	11.02	—	—
	Seed × varieties	3	295.23	98.41	2.15	3.10
	Error (b)	20	914.25	45.71		
	Total	24	1220.50			

Summary of Results (Percentage of Plants Emerged)

Variety	1932		1933		1934	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
Mindum	—	—	—	—	68.3	73.5
Marquis	—	—	67.3	50.2	—	—
Ceres	—	—	66.3	69.3	—	—
Reward	—	—	56.7	56.3	—	—
Pentad	74.8	71.8	58.8	64.7	70.0	63.8
Acme	69.2	66.7	47.0	46.5	68.5	63.0
Pentad × Marquis (R.L. 726)	74.0	67.2	63.2	54.8	70.0	76.2
Pentad × Marquis (R.L. 723)	72.0	70.1	—	—	—	—
Mean*	72.5	69.0	59.9	57.0	69.2	69.1
Standard error of varieties	—	—	3.11	3.11	—	—

** The difference between means of healthy and diseased seed not significant in 1932, 1933, and 1934*

The Effect of Kernel Smudge on the Development of Seedling Blight and Root Rot

As pointed out in the preceding section of this paper, most investigations with discolored seed of cereals have shown that, in general, such seed gives rise to diseased seedlings. The results of some, however, are not in accord with this finding. In the present studies it has been demonstrated (Table VI) that, under field conditions in Manitoba during 1932, 1933, and 1934, the differences in plant emergence between healthy and discolored seed were not statistically significant. The results of preliminary tests (Table II) indicate that the conflicting results obtained by earlier workers may easily be accounted for by the supposition that the seed used by some was infected with *Alternaria* while that used by others was infected with *H. sativum*. It has been clearly demonstrated (Table III) that the amount of seedling disease resulting from kernel smudge depends upon the fungus with which the seed is infected.

Further information concerning the effect of planting discolored seed of wheat on the incidence of seedling blight and root rot was obtained from the field experiments of 1932, 1933, and 1934. The summarized results of these experiments are given in Table VII.

The differences observed in the amount of root rot in these years between plots planted with discolored seed and adjoining plots planted with healthy seed were not statistically significant. There was, however, slightly more disease in the plots planted with diseased seed. The discolored seed used in these experiments was infected with *Alternaria* spp. From the results presented in Table VII, it may be concluded that seed attacked by kernel smudge of the *Alternaria* type does not produce root rot in the subsequent crop.

The Effect of Kernel Smudge on Yield

It has been demonstrated by a number of workers (3, 8, 10, 14, 20, 35, 40, 41, 45, 51) that wheat seed infested by *H. sativum* produced fewer plants than healthy seed. These plants were usually more affected by root rot than were plants from healthy seed. On the other hand, Dastur (12) showed that infection of wheat seed by this fungus was not always a determining factor influencing crop yield. He pointed out that the amount of root rot developed on the plants was determined to a great extent by the weather conditions which prevailed during the early stages of plant growth. Stening (48), Miège (29), and Waldron (50) showed that the planting of discolored seed did not influence the yield of wheat.

In the present investigations an effort was made to determine whether, under field conditions, the planting of seed affected by the *Alternaria* type of kernel smudge affected the yield. The yield data, given in Table VIII, were taken from the field experiments made at Winnipeg in 1932, 1933, and 1934. They show that in 1932 the yield of plants from healthy seed was significantly higher (4.0 bushels per acre) than the yield of plants from diseased seed. In 1933 and 1934, the differences in yield observed between healthy

TABLE VII

EFFECT OF PLANTING HEALTHY AND DISEASED (KERNEL SMUDGE) SEED OF WHEAT IN FIELD PLOTS IN 1932, 1933 AND 1934 ON THE INCIDENCE OF ROOT ROT

Analyses of Variance

Year	Source of variance	Degrees of freedom	Sum of squares	Mean square	F	5% point
1932	Replicates	5	901.85	108.37		
	Varieties	3	159.23	53.07	1.07	3.29
	Error (a)	15	740.90	49.39		
	Total	23	1801.98			
	Seed (healthy and diseased)	1	63.02	63.02	2.67	4.35
	Seed × varieties	3	98.73	32.91	—	—
	Error (b)	20	470.75	23.53		
	Total	24	632.50			
1933	Controlled error	8	1004.15	125.52		
	Varieties	5	1443.71	288.74	9.46	2.66
	Error (a)	22	671.08	30.50		
	Total	35	3118.94			
	Seed (healthy and diseased)	1	85.80	85.80	2.73	4.17
	Seed × varieties	5	573.07	114.61	3.64	2.53
	Error (b)	30	943.99	31.46		
	Total	36	1602.86			
1934	Controlled error	8	1202.85	150.30		
	Varieties	3	805.94	268.64	3.98	3.49
	Error (b)	12	810.05	67.50		
	Total	23	2818.84			
	Seed (healthy and diseased)	1	14.08	14.08	—	—
	Seed × varieties	3	238.72	79.57	2.54	3.10
	Error (b)	20	625.64	31.28		
	Total	24	878.44			

Summary of Results (Disease Rating)

Variety	1932		1933		1934	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
Mindum	—	—	—	—	27.2	22.0
Marquis	—	—	55.5	68.6	—	—
Ceres	—	—	56.8	54.9	—	—
Reward	—	—	64.2	64.8	—	—
Pentad	51.6	51.1	63.9	59.3	28.8	33.2
Acme	54.0	57.6	70.3	71.8	27.2	31.2
Pentad × Marquis (R.L. 726)	49.8	56.1	60.8	65.3	20.2	19.6
Pentad × Marquis (R.L. 723)	51.5	51.1	—	—	—	—
Mean*	51.6	53.6	61.9	64.1	25.3	26.5
Standard error of varieties	—	—	2.25	2.25	3.55	3.35

** The difference between means of healthy and diseased seed not significant in 1932, 1933, and 1934.*

TABLE VIII

EFFECT OF PLANTING HEALTHY AND DISEASED (KERNEL SMUDGE) SEED OF WHEAT IN FIELD PLOTS IN 1932, 1933 AND 1934 ON YIELD

Analyses of Variance

Year	Source of variance	Degrees of freedom	Sum of squares	Mean square	F	5% point
1932	Replicates	5	913.38	182.67		
	Varieties	3	667.40	222.46	16.20	3.29
	Error (a)	15	326.95	21.79		
	Total	23	1907.73	426.92		
	Seed (healthy and diseased)	1	137.70	137.70	10.00	4.35
	Seed × varieties	3	102.68	34.22	2.48	3.10
	Error (b)	20	275.57	13.77		
	Total	24	515.95			
1933	Controlled error	8	327.27	40.90		
	Varieties	5	619.84	123.96	13.64	2.66
	Error (a)	22	199.98	9.09		
	Total	35	1147.09			
	Seed (healthy and diseased)	1	2.45	2.45	1.75	4.17
	Seed × varieties	5	4.43	0.89	—	—
	Error (b)	30	42.01	1.40		
	Total	36	48.89			
1934	Controlled error	8	3123.94	390.49		
	Varieties	3	3178.41	1059.47	202.19	3.49
	Error (a)	12	62.88	5.24		
	Total	23	6365.23			
	Seed (healthy and diseased)	1	44.66	44.66	—	—
	Seed × varieties	3	9.88	3.29	—	—
	Error (b)	20	1262.19	63.11		
	Total	24	1316.73			

Summary of Results (Yield in Bushels per Acre)

Variety	1932		1933		1934	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
Mindum	—	—	—	—	22.2	26.3
Marquis	—	—	14.4	13.5	—	—
Ceres	—	—	17.0	16.9	—	—
Reward	—	—	8.0	7.0	—	—
Pentad	52.1	45.3	12.7	13.1	41.1	42.2
Acme	51.6	45.8	13.1	13.0	44.4	46.1
Pentad × Marquis (R.L. 726)	41.5	41.4	10.8	10.3	29.7	32.8
Pentad × Marquis (R.L. 723)	46.7	43.3	—	—	—	
Mean	47.9	43.9	12.6	12.3	34.3	36.8
Standard error of mean*	0.76		—		—	
Standard error of varieties	1.90	1.90	1.23	1.23	0.93	0.93

** The difference between means of healthy and diseased seed not significant in 1933 and 1934.*

and diseased seed were not significant. From these results it may be concluded that the planting of discolored seed, in which the disease is due to species of *Alternaria*, does not, in general, materially influence the yield.

The Effect of Planting Discolored Seed on the Occurrence of Kernel Smudge in the Subsequent Crop

Little definite information concerning the influence of planting discolored seed on the occurrence of kernel smudge in the subsequent crop is available. Stening (48) stated that discolored seed of wheat could be safely planted. There appeared to be little probability that sowing diseased seed had any direct influence on the amount of kernel smudge in the harvested grain. Dastur (12) showed that the amount of discoloration in the grain is influenced more by atmospheric temperature and moisture than by any other factor, including the planting of diseased seed.

To secure more definite information concerning the effect of planting discolored seed on the amount of disease occurring in the subsequent crop, an examination was made of the threshed grain of the field experiments of 1932, 1933, and 1934. In each of these years, the seed planted in the field tests was obtained from the crops of the preceding year. After the plants in the various plots had been harvested and the grain threshed, the percentage of discolored kernels present in each plot sample, based on a count of a 1000 kernels, was computed. The results of the three-year test, showing the percentage of discolored kernels in the grain of plants arising from healthy and discolored seed, are given in Table IX.

In 1932 there was no difference in the amount of kernel smudge in the crop resulting from healthy and diseased seed, but in 1933 and 1934 slightly more diseased kernels were found in the grain from discolored than from healthy seed. Only in 1934, however, was this difference statistically significant. It may be concluded from these results that planting seed infected with kernel smudge of the *Alternaria* type does not result in a material increase in the amount of disease in the grain of the next crop. To this extent, therefore, these results are in agreement with those of Stening (48).

The Relative Effect of the *Alternaria* and *Helminthosporium sativum* Types of Kernel Smudge in Pentad Wheat on Plant Emergence, Occurrence of Root Rot, and Yield

Discolored kernels in a number of wheat varieties grown in Manitoba during the years 1929 to 1934 yielded more or less consistently cultures of *Alternaria* species. Moreover, field tests (Tables VI–IX) showed that kernel smudge of this type caused little or no harm to the subsequent crop. These results led to the conclusion that *Alternaria* was the main cause of kernel smudge in wheat grown in Manitoba. In 1935, however, the results obtained at Winnipeg forced the writers to modify this conclusion. During the latter part of the summer of that year, spores of *H. sativum*, in greater numbers than in any of the three previous years, appeared in Manitoba (Table IV).

TABLE IX

EFFECT OF PLANTING HEALTHY AND DISEASED (KERNEL SMUDGE) SEED IN FIELD PLOTS ON THE AMOUNT OF KERNEL SMUDGE IN THE CROPS OF 1932, 1933 AND 1934

Analyses of Variance

Year	Source of variance	Degrees of freedom	Sum of squares	Mean square	F	5% point
1932	Replicates	5	382.21	76.44		
	Varieties	3	17503.70	5834.56	153.53	3.29
	Error (a)	15	570.63	38.04		
	Total	23	18456.54			
	Seed (healthy and diseased)	1	0.10	0.10	—	—
	Seed × varieties	3	116.52	38.84	3.66	3.10
	Error (b)	20	211.71	10.58		
	Total	24	328.33			
1933	Controlled error	8	79.01	9.88		
	Varieties	5	19158.16	3831.63	506.10	2.66
	Error (a)	22	166.55	7.57		
	Total	35	19403.72			
	Seed (healthy and diseased)	1	9.32	9.32	—	
	Seed × varieties	5	78.56	15.71	—	—
	Error (b)	30	288.70	9.62		
	Total	36	376.58			
1934	Controlled error	5	1513.88	302.62		
	Varieties	3	49296.03	16432.01	214.01	3.29
	Error (a)	15	1151.77	76.78		
	Total	23	51960.88			
	Seed (healthy and diseased)	1	305.02	305.02	26.45	4.35
	Seed × varieties	3	124.43	41.48	3.60	3.10
	Error (b)	20	230.66	11.53		
	Total	24	660.11			

Summary of Results (Percentage of Smudged Kernels)

Variety	1932		1933		1934	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
Mindum	—	—	—	—	24.2	27.9
Marquis	—	—	0.8	0.7	—	—
Ceres	—	—	0.9	1.8	—	—
Reward	—	—	0.6	0.7	—	—
Pentad	42.2	37.3	41.3	40.7	79.0	86.0
Acme	39.0	42.8	25.4	30.8	69.0	77.9
Pentad × Marquis (R.L. 726)	2.3	2.3	1.2	0.7	5.4	5.9
Pentad × Marquis (R.L. 723)	1.6	2.4	—	—	—	—
Mean	21.2	21.2	11.7	12.5	44.4	49.4
Standard error of mean*	—		—		0.69	
Standard error of varieties	2.52	2.52	1.12	1.12	3.57	3.57

* *The difference between healthy and diseased seed not significant in 1932 and 1933.*

When the field plots were harvested, a large percentage of the kernels in the threshed grain were found to be discolored. Isolations were then made from discolored seed of Pentad wheat grown in 1934 and 1935. The results (Table II) showed distinctly that in 1934, as in the previous years, the kernels were infected mostly with *Alternaria*, while in 1935 the seed was infected almost entirely with *H. sativum*.

A preliminary greenhouse test (Table III) showed that there was a marked difference in the amount of seedling blight that developed in the seedlings resulting from the two types of seed. The seedlings from the 1935 seed were severely blighted while those from the 1934 seed were not. In 1936, in order to clarify the greenhouse results by means of a field test, the seed of the 1934 and 1935 crops of Pentad wheat was tested in properly randomized and replicated field plots at Winnipeg. The results of this experiment are given in Table X.

TABLE X

THE EFFECT OF THE *Alternaria* AND *H. sativum* TYPES OF KERNEL SMUDGE ON EMERGENCE OF SEEDLINGS, SEVERITY OF ROOT ROT, AND YIELD OF PENTAD WHEAT UNDER FIELD CONDITIONS IN 1936

Crop year	Dominant fungus in seed	Seedling emergence, %	Disease rating (seedling)	Disease rating (adult plants)	Yield per acre, bu.
1934	*Alternaria* spp.	87.5	28.5	53.1	29.2
1935	*H. sativum*	24.9	80.6	85.3	20.6
Difference		62.6	52.1	32.2	8.6

Table X shows distinctly that the *H. sativum* type of kernel smudge (1935 seed) caused considerably more seedling blight and root rot in the crop than did the *Alternaria* type of this disease (1934 seed). Thus there is little reason to doubt that the disagreements in the findings of previous workers have been due to the fact that the seed which these workers had used was infected by at least two different kinds of fungi, one of which was pathogenic to the growing plants, while the other was not. The similarity in symptoms produced by *Alternaria* and *H. sativum* in the seed of cereals has, no doubt, led many of these workers to believe that they were dealing with a disease produced by a single fungus.

Control

The literature dealing with the control of kernel smudge in grain and the prevention of adverse consequences to the next crop is not very extensive. Bolley (5) advised crop rotation as a means of controlling this disease, because he found seed discolorations very prevalent in cereals grown on "wheat-sick" soil. McKinney (26) made similar recommendations. Christensen (9), Goulden (17), Miège (29), and Weniger (51) showed that wheat varieties differed in susceptibility to the disease. Peterson* showed that these differences were heritable, thereby suggesting that varieties of wheat resistant to kernel smudge may be produced.

* *See reference, page 91.*

With the exception of the work of Bolley (5) and McKinney (26), no attempts have been made to prevent the development of kernel smudge in cereal crops. A few workers, however, have attempted to control the disease by destroying the fungus within the discolored seed. Atanasoff and Johnson (1) used dry heat for this purpose. Peyronel (34) reported that the practice of treating diseased seed with copper fungicides was extensively followed in Sicily, and suggested that good results might follow the use of copper dusts for this purpose. Waldron (50) attempted to prevent seedling blight, and to increase emergence of seedlings arising from discolored seed, by treating such seed with New Improved Ceresan. This method of control did not appear satisfactory.

The Effect of Sulphur Dust Applied to the Growing Plants

At Winnipeg, an attempt was made to control kernel smudge by dusting the growing crop with sulphur dust. Grain samples from field plots of Marquis wheat, which received, during the growing seasons of 1929, 1930, and 1931, periodic dustings with finely divided sulphur to prevent stem rust, contained in the latter two years a smaller percentage of discolored kernels than did grain samples from plots that received no dust (Table XI).

TABLE XI

The effect of dusting growing plants with sulphur on the percentage of kernel smudge in Marquis wheat. (Percentages based on 1000-kernel counts)

Year	Treatment	Amount of dust, lb. per acre	Dusting interval, days	Total dustings	Percentage of kernel smudge
1929	None	0	—	0	0.3
	Dusted	45	3	9	0.5
1930	None	0	—	0	4.9
	Dusted	15	21	3	1.7
	Dusted	15	14	5	1.8
	Dusted	15	7	9	1.2
	Dusted	15	3	15	0.9
	Dusted	30	14	3	1.1
	Dusted	30	7	5	1.1
	Dusted	30	3	9	0.5
1931	None	0	—	0	0.9
	Dusted	30	3	15	0.6

The results in Table XI indicated that dusting growing plants with sulphur controls kernel smudge to some extent. However, as Marquis wheat was not very susceptible to kernel smudge under Manitoba conditions in 1929, 1930, and 1931, further tests with other varieties of wheat seemed desirable. In 1932, therefore, sulphur-dusting tests were designed in which the rust-resistant hybrid, Pentad × Marquis (R.L. 730), was used. Unfortunately, this variety also proved to be somewhat resistant to kernel smudge, so that, in 1933, 1934 and 1935, the variety Pentad, which is resistant to stem rust in Manitoba but very susceptible to kernel smudge, was substituted.

The experiments from which the data shown in Table XI were taken were not specially designed to give information concerning kernel smudge. In the following years, however, a suitable plan of plot experiment was used. The seed was planted in rod rows, one foot apart, by means of a drill. After the plants had reached the shot blade stage of growth, pairs of rows, which were separated from pairs of control rows (undusted), were dusted with sulphur at the rate of 30 lb. per acre per application, at intervals of four days until the plants were practically mature. The undusted rows were protected from dust drift by three buffer rows. After the crop had been harvested and threshed, the percentage of discolored kernels, based on a count of 1000 kernels taken at random from the grain of each row, was calculated. The results of these tests, showing the percentage of smudged kernels from dusted and undusted plots of Pentad wheat, are given in Table XII.

TABLE XII

THE EFFECT OF DUSTING GROWING PLANTS OF PENTAD WHEAT WITH SULPHUR ON THE DEVELOPMENT OF KERNEL SMUDGE OF WHEAT DURING THE FOUR YEARS 1932 TO 1935

Treatment	Percentage of smudged kernels			
	1932	1933	1934	1935
Dusted	2.4	24.1	48.5	91.9
Undusted	3.3	27.3	51.5	91.0
Difference	0.9	3.2	3.0	− 0.9
Value of t*	3.07	4.24	3.53	1.79

* *5% point for value of* $t = 1.95$.

Table XII shows that dusting the growing plants with sulphur reduced the disease by a significant degree in 1932, 1933, and 1934, but not in 1935. In no year, however, was the reduction sufficient to justify the use of this method of control.

THE EFFECT OF SEED TREATMENT

During the years 1932 to 1935 laboratory and greenhouse tests were made to determine the effect, on the control of seedling blight and root rot in the seedlings, of disinfecting seed infected with kernel smudge fungi with various chemicals. In these tests, mercuric bichloride steep, Semesan steep, copper carbonate dust, Ceresan and New Improved Ceresan were used. The results with seed produced in 1932, 1933, and 1934 were mostly negative, or at least inconclusive. That is to say, seed treatment did not give an increase in seedling emergence or an appreciable reduction in the amount of root rot. The reason for this seemed to be that *Alternaria* was the dominant fungus in the seed produced in these years, so that little or no disease developed in the seedlings, even in the controls.

TABLE XIII

EFFECT OF TREATING PENTAD WHEAT SEED, NATURALLY INFECTED WITH KERNEL SMUDGE IN 1935, WITH NEW IMPROVED CERESAN, LEYTOSAN, AND COPPER CARBONATE DUSTS, ON PLANT EMERGENCE, INCIDENCE OF ROOT ROT, AND YIELD IN 1936

Analyses of Variance

Data	Source of variance	Degrees of freedom	Sum of squares	Mean square	F	5% point
Plant emergence	Replicates	5	726.89	145.37		
	Fungicides	2	13676.44	6838.22	210.47	3.88
	Error (a)	12	389.95	32.49		
	Seed (dusted and undusted)	1	8040.11	8040.11	137.43	4.54
	Error (b)	15	877.50	58.50		
	Total	35	23710.89			
Disease rating	Replicates	5	445.83	89.16		
	Fungicides	2	7340.41	3670.20	272.27	3.88
	Error (a)	12	161.78	13.48		
	Seed (dusted and undusted)	1	4489.00	4489.00	111.61	4.54
	Error (b)	15	603.36	40.22		
	Total	35	13040.38			
Yield	Replicates	5	32.53	6.50		
	Fungicides	2	68.96	34.48	7.32	3.88
	Error (a)	12	56.52	4.71		
	Seed (dusted and undusted)	1	218.05	218.05	24.09	4.54
	Error (b)	15	135.77	9.05		
	Total	35	511.83			

Summary of Results

Data	Seed treatment	New Improved Ceresan	Leytosan	Copper carbonate	Standard error
Emergence (per cent)	Dusted	84	89	29	±2.33
	Undusted	38	39	38	±2.33
	Difference	+46	+50	− 9	
	Standard error	± 3.12	± 3.12	± 3.12	
Disease rating*	Dusted	40	37	81	±1.50
	Undusted	75	74	76	±1.50
	Difference	−35	−37	+ 5	
	Standard error	± 2.58	± 2.58	± 2.58	
Yield (bu. per acre)	Dusted	19.2	17.9	14.5	±0.89
	Undusted	12.6	10.6	13.7	±0.89
	Difference	+ 6.6	+ 7.3	+ 0.8	
	Standard error	± 1.23	± 1.23	± 1.23	

* *Computed on the basis of number of seeds sown; highest numerical rating given for non-emergence.*

In the autumn of 1935, when seed infected and discolored by *H. sativum* was available, greenhouse tests with the diseased seed showed that disinfection with two commercial dusts, New Improved Ceresan and Leytosan, resulted in a marked control of seedling blight. From these tests it appeared that both fungicides were lethel to the mycelium within the seed coats, as well as to surface-borne spores.

In 1936, a comprehensive field experiment was made in which three dusts (New Improved Ceresan, Leytosan, and copper carbonate) were used to treat discolored seed of Pentad wheat from the 1935 crop. Table XIII gives the complete analyses of variance of the experimental data, and presents a summary table showing the effect of the treatments on seedling emergence, severity of the root-rot disease, and on yield. The data show that, while treatment with copper carbonate was not beneficial, treatment with New Improved Ceresan and Leytosan improved the stand, reduced root rot and increased yield. An increase of about seven bushels per acre was obtained in 1936 as the result of treating Pentad seed, infected with *H. sativum*, with the two organic mercurials.

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THE RELATION BETWEEN BACTERIAL NUMBERS AND BIOCHEMICAL VALUES IN MILK FROM STREPTOCOCCUS-FREE QUARTERS[1]

BY C. K. JOHNS[2] AND E. G. HASTINGS[3]

Abstract

An attempt has been made to determine whether abnormalities in the composition of the milk from streptococcus-free quarters are due to bacterial infection.

Four normal young animals whose previous history was known in considerable detail were selected for study. Series of samples from consecutive milkings were examined at intervals over a period of several months. Unfortunately none of the quarters studied showed more than a transitory rise in the level of catalase values during these detailed studies, so that the findings reported do not settle the question. Regular rhythmic fluctuations in counts from the foremilk were again noted, extremely high counts for the morning milkings alternating with low counts for the afternoon milkings.

The relation between bacterial numbers and abnormal composition of the foremilk is not a simple one. High biochemical values may accompany low count levels while extremely high counts may exert no apparent influence upon the composition of the milk at that time. Possibly a second agent in addition to bacteria is concerned.

More intensive studies of a single animal yielding milk with very high counts revealed higher biochemical values for strippings than for foremilk, those for catalase being surprisingly high. Despite the unusual nature of these findings, none of the four quarters became definitely abnormal during the fourteen weeks during which this animal was under observation, but subsequent routine samplings have shown that all four quarters must now be regarded as abnormal.

No one type of organism appears to be associated with changes in the composition of the secretion.

While the secretion yielded by cows infected with *Str. agalactiae* generally shows high values for chlorides, catalase and pH, similar abnormal secretions from significant numbers of streptococcus-free* heifers have recently been reported by several workers (1, 3, 7). Since these "indirect" tests are widely used as diagnostic agents in attempts to control chronic mastitis, such findings suggest the need for extreme caution in their use.

Although the workers cited have all sought for the presence of some causative organism or organisms which might serve to explain the abnormal composition of the secretion, the search has so far been unsuccessful. Rarely

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* *In the light of the findings recently reported by Hucker (2) the term "streptococcus-free" indicates that mastitis streptococci were at no time demonstrated to be present in the milk.*

does one encounter a quarter free from streptococci giving abnormal milk where a single bacterial species is constantly present in significant numbers. Too often the flora, both of foremilk and of strippings, is highly miscellaneous. The predominant types are various species of micrococci apparently identical with those occurring in milk consistently normal in composition.

It has previously been pointed out by one of us (3) that in at least one of the animals studied, there appeared to be some relation between total bacterial numbers and abnormal secretion. In the heifer in question the appearance of abnormal biochemical values (high chlorides, catalase and pH) in one quarter was preceded by a definite rise in the general level of bacterial counts. The studies to be described here deal with attempts to determine whether this always holds true or whether, as Wolf (9) maintains, the marked increase in bacterial numbers is due to the more favorable conditions furnished by the changed composition of the secretion.

It has been pointed out in a previous paper (3) that the most satisfactory picture of the condition of a quarter is obtained by the study of a series of samples taken at consecutive milkings. This method was followed in the present studies with animals carefully selected on the basis of their previous history.

Part I

Periodical examinations of milk from four normal heifers

Four animals, all in the first half of their second lactation period, were selected for study. Samples of foremilk from these animals had been examined* at fortnightly intervals from their first calving up to the commencement of the present studies. In addition, at one milking each week, the milk from each individual quarter was collected separately by the use of a specially constructed milking machine. Details concerning the yield from individual quarters will be given as each animal is discussed.

Sampling

Commencing March 11, 1937, samples were obtained at five consecutive milkings. Three other similar series of samplings were conducted at short intervals, the last one commencing on April 11.

After discarding the first few streams, approximately 30 cc. of foremilk were drawn into a sterile container. No special precautions as to cleaning the udder and teats were employed. Samples were removed to the laboratory at once in the case of the afternoon milkings; approximately one hour elapsed at the morning milkings before the analysis commenced.

Analysis

Plate counts were made on a medium containing agar 2%, tryptone 0.5% and skim milk 1%, this medium having previously been found to be superior to veal infusion agar. Plates were counted after incubation for 48 hr. at 37° C.

* *These examinations were made by Dr. E. H. Peterson, to whom the authors wish to make acknowledgment for his courtesy in making his data available.*

The catalase test was relied upon to indicate the relative normality of the milk from the quarters being studied, previous experience having shown it to be more satisfactory than the chloride or pH determinations (4). The modification devised by one of us (E.G.H.), in which the oxygen liberated is trapped beneath an agar seal in a long glass tube, was employed. Readings were made after 24 hours at room temperature (approximately 20-25° C.). On the basis of previous findings, a value in excess of 40% of gas expressed as a percentage of the volume of milk used in the test was considered to be definitely abnormal.

Findings

Heifer No. 3. This animal began her second lactation period on October 31, 1936. No abnormalities were noted in the previous lactation period or up to the beginning of the present studies. During the first lactation period the left hind quarter yielded slightly less milk than the right hind, while the same has held true during the present lactation period. In addition, during the present period the left front quarter has yielded significantly less than the right front quarter.

Data obtained are presented graphically in Fig. 1*. Normal values for catalase were encountered at each of the twenty milkings, samples from the right hind quarter excepted. The single high count on the left front quarter on March 12, a.m., appears to have been without influence upon the catalase values.

The right hind quarter, on the other hand, furnished samples with rather high catalase values during the second period of sampling (March 22 to 24). As the values had returned to normal by the following week it seems probable that the temporary rise in catalase values was associated with some physical injury. In view of the contention of Wolf (9) that abnormally high bacterial numbers are the result, rather than the cause, of abnormal secretion, it is of interest to note that in this case the counts remained low during and subsequent to the period when the catalase values were high.

There is nothing in either catalase values or counts to indicate any relationship between them and the relative milk production per quarter.

Heifer No. 7. During the first lactation period of this heifer, no consistently high values were noted, although the left front quarter occasionally yielded a sample with a high catalase value. Values for the left hind quarter were generally slightly higher than those for the other three quarters. No marked differences in production were noted for the four quarters. Nine days after the birth of the second calf on October 20, 1936, the milk was normal. Early in January 1937 the right front quarter suffered an acute attack of mastitis due to an infection with a coliform organism; at each subsequent sampling high values for catalase and chlorides were noted. The yield of milk from this quarter showed a sharp decline as a result of this attack. The right hind quarter yielded about 25% more milk than the left hind during the present lactation period.

**Values for the afternoon milkings appear on the dotted lines on all figures.*

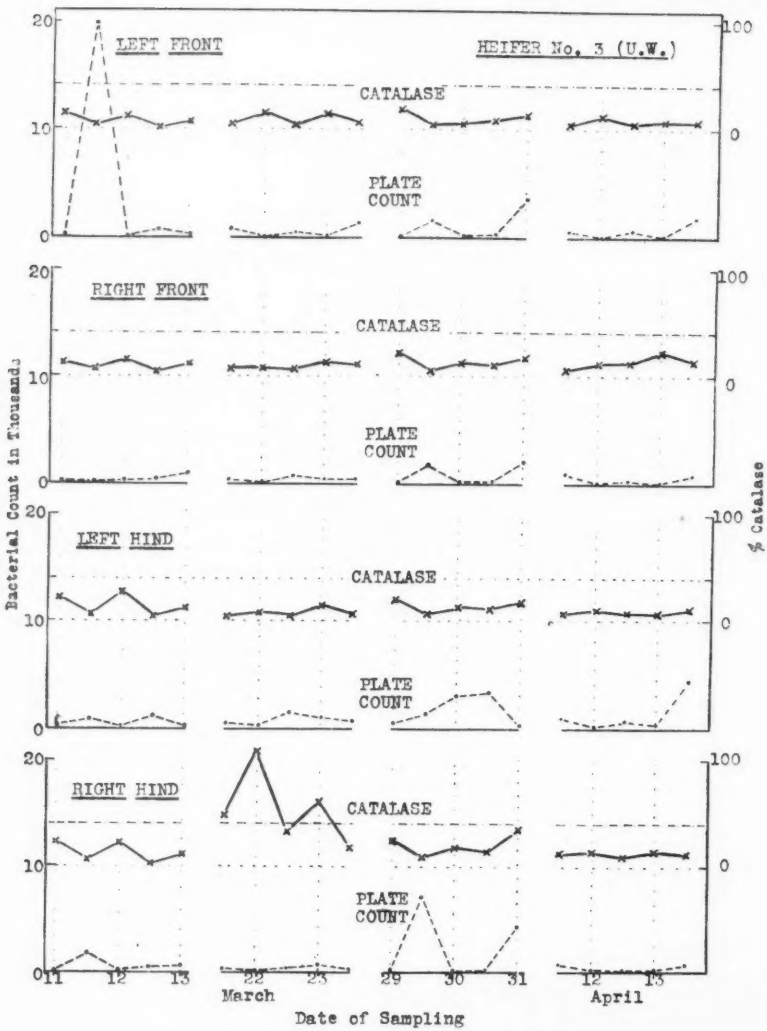

FIG. 1. *Data on foremilk samples, Heifer No. 3, (U.W.)*

From the data presented in Fig. 2 it will be observed that the influence of the acute attack of mastitis in the right front quarter was still being felt, although the general level of counts was comparable with those for the remaining quarters. The coliform organism was still present, the numbers fluctuating markedly from milking to milking. During the second period of sampling (March 22 to 24) there was a "flare-up" of the previous infection, large

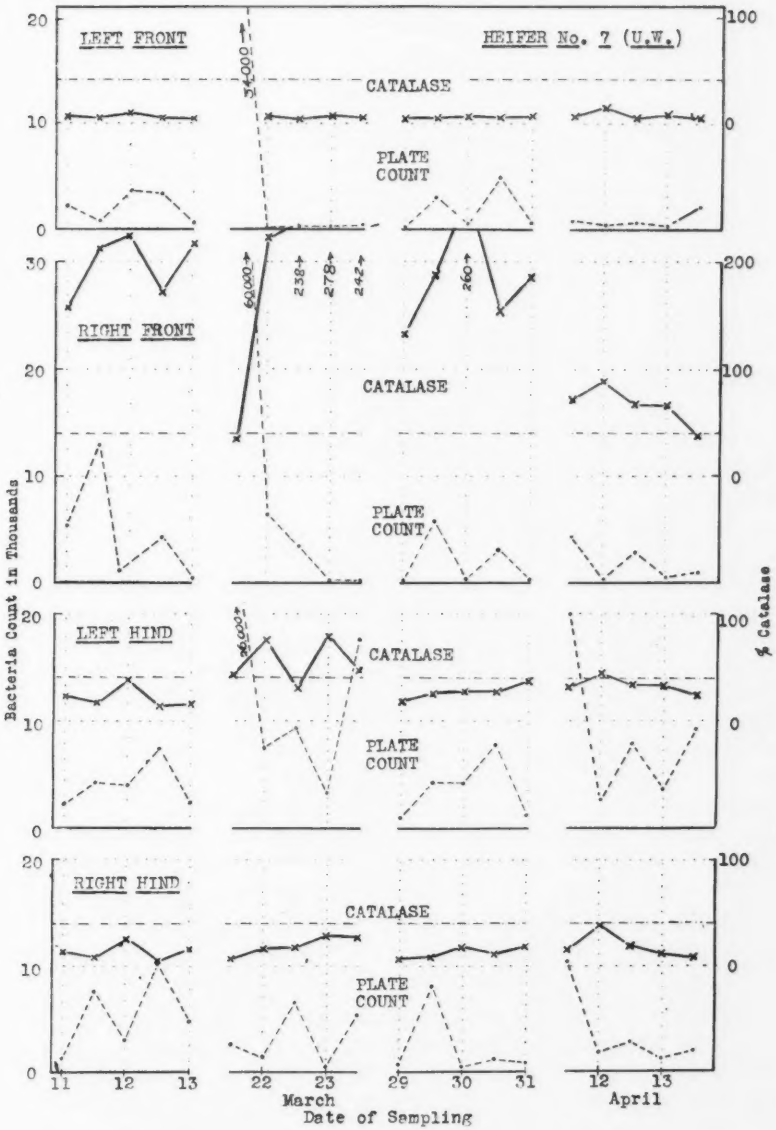

FIG. 2. *Data on foremilk samples, Heifer No. 7, (U.W.)*

numbers of the organism concerned being present in the sample drawn on the morning of March 22. The quarter was hard and the milk obviously abnormal. The catalase values rose sharply at this time, remaining at a very high level for several milkings after the count had declined. High catalase values accompanied by reasonably low counts appeared during the following week, while in the final series (April 12 to 14) the values were significantly lower, although still above the normal limit.

No values in excess of 40% were obtained for samples from the left front and right hind quarters but the left hind quarter showed a somewhat higher level of values throughout. During the second period of sampling, four of the five samples from this quarter were above the normal limit, while the level of counts was also definitely higher. It will be recalled that this quarter showed somewhat higher values for catalase during the first lactation period. Whether these higher levels were "normal" for this quarter or whether there was some slight disturbance of function cannot be decided on the basis of the information at hand.

It will be observed that bacteria counts were almost always higher for the morning samples while catalase values were generally higher at the afternoon milkings.

Excluding the definitely infected right front quarter, it appears that among the other three quarters the level of catalase values is in fairly close agreement with the level of bacteria counts. The occurrence of reasonably low counts concurrently with extremely high catalase values in the case of the right front quarter may serve to explain the finding of abnormal secretion from apparently normal udders where the bacteria counts are lower than might be expected. Such cases may be due to the presence of large numbers of bacteria in the udder at some previous time.

Heifer No. 10. No consistent abnormalities were noted during the first lactation, although the right front quarter suffered from an acute attack of mastitis on August 20, 1936, at which time a Gram-positive streptobacillus was isolated. Five days later the organism was no longer present in the milk and the composition rapidly returned to normal. Milk yields per quarter were comparable throughout the lactation period.

This animal commenced her second lactation period on November 23, 1936. A single abnormal catalase value of 90% was noted for the right hind quarter on January 3, but this was followed by normal values.

From the data presented in Fig. 3, it will be noted that the general levels of catalase values for the right front and right hind quarters were somewhat higher than those for the remaining quarters, with an occasional value in excess of 40%. Since the production by the right front quarter was comparable to that by the left front, while the right hind quarter yielded about one-fifth less than the left hind, there seems to be no clear connection between the level of catalase values and yield of milk.

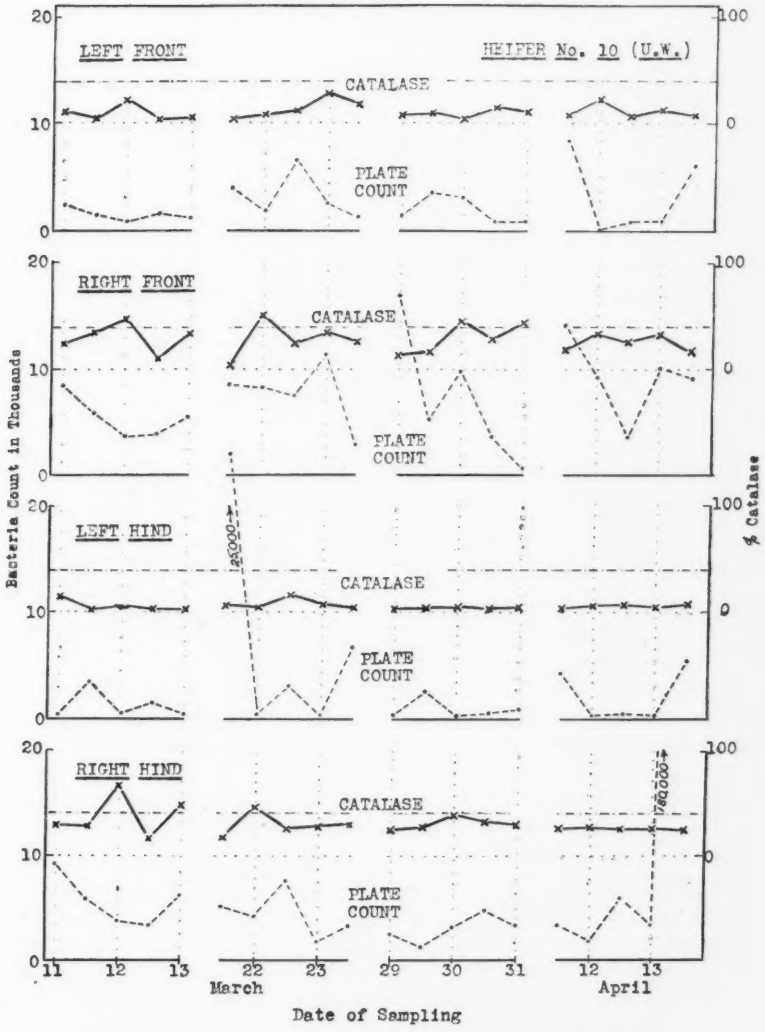

FIG. 3. *Data on foremilk samples, Heifer No. 10, (U.W.)*

As in the case of heifer No. 7, there is a fairly close agreement between the levels of catalase values and of bacteria counts for the various quarters.

Heifer No. 12. During the first lactation period of this heifer the two front quarters showed slightly higher values for chlorides and catalase than the

hind quarters, those for the right front quarter being the more abnormal. The right hind quarter produced over 20% more milk than the corresponding quarter during this period.

The second freshening occurred on February 8, 1937. Fairly normal values were encountered a week later, and these continued up to the time of the present studies. The right hind quarter is again producing more milk than the left hind quarter.

The unusually high counts obtained made it necessary in graphing the data to employ a scale in which each unit represents five times as many bacteria as those used for the data concerning the other three heifers. This should be borne in mind when comparing the data presented in Fig. 4 with those in the three preceding figures. In addition to the high counts, samples from all four quarters were characterized by a highly miscellaneous flora, no single type appearing in sufficient numbers to attract attention. Micrococci were most common, although tiny Gram-negative cocco-bacilli were occasionally isolated from all four quarters.

No abnormal catalase values were encountered, except from the left hind quarter upon two occasions. The general level of values for this quarter was definitely higher than those for the remaining three, while extremely high counts were obtained upon three occasions during the last two sampling periods. The enormous fluctuations in counts from milking to milking, with counts higher at the morning milkings, exhibit a regular rhythmic fluctuation similar to that previously noted in a number of other animals (4). Similar but less marked fluctuations were observed for the other three quarters.

The general high level of counts, with occasional extremely high counts, without any marked influence upon the catalase values, suggested the advisability of investigating the condition of the strippings. Accordingly, samples of both foremilk and strippings were obtained at the last three milkings (April 13 and 14) and analyzed for catalase, chlorides, pH and total count. Unfortunately, in five instances the sample of strippings was not large enough to enable all of these tests to be made. The data obtained appear in Table I. The contrast between the catalase values for the strippings and foremilk samples is most surprising. (In only one other animal whose foremilk and strippings have been studied has anything resembling this condition been encountered. In this case three of the four quarters were definitely abnormal; two of these and the remaining normal quarter all showed somewhat higher values for catalase on the strippings). While the pH values for strippings were all slightly higher than those for foremilk, the differences were of much smaller magnitude, with only two in excess of pH 6.8. Differences in chloride content were even smaller. These data, together with the occasional very high counts on strippings samples, indicated that the bacteria were not confined to the lower portions of the udder as in the cases reported by Little and Foley (6). It was believed that this animal was worthy of further study, conse-

quently further periodical samplings were conducted over the period May 3 to June 16. These are reported upon in the following section.

TABLE I

DATA ON SAMPLES OF FOREMILK AND STRIPPINGS
(HEIFER NO. 12 (U.W.) APRIL, 1937)

——	Left front	Right front	Left hind	Right hind
Counts				
13th a.m. F	3,700	2,520	145,000	4,500
S	630	9,200	30	640
p.m. F	460	1,680	1,700	2,600
S	21,100	900,000	1,720	90
14th a.m. F	5,500	160,000	160,000	56,400
S	9,800	5,200	910	6,000
Catalase				
13th a.m. F	11	6	27	5
S	178	122	184	81
p.m. F	15	14	26	13
S	157	—	251	55
14th a.m. F	10	9	21	10
S	—	133	79	59
pH				
13th a.m. F	6.46	6.52	6.53	6.50
S	6.79	6.61	6.68	6.68
p.m. F	6.55	6.60	6.60	6.57
S	6.70	—	6.86	6.62
14th a.m. F	6.56	6.56	6.56	6.56
S	6.93	6.76	6.67	6.67
Chlorides				
13th a.m. F	0.101	—	0.100	—
S	0.120	—	0.095	0.112
p.m. F	0.100	0.105	0.105	0.097
S	0.119	—	—	0.100
14th a.m. F	—	0.125	0.115	0.105

F—Foremilk. S—Strippings.

Part II

FURTHER DETAILED STUDIES WITH HEIFER NO. 12 (U.W.)

The unusual nature of the findings reported for this heifer suggested that further detailed studies of this animal might be more profitable than a continuation of those described in Part I. Arrangements were therefore made to analyze samples of both foremilk and strippings from five consecutive milkings at several periods during April, May and June, 1937. Determinations were made of chlorides and pH in addition to catalase and plate counts.

Methods

The collection of samples, plating, and catalase determinations were conducted in the manner previously described. Chlorides were determined by titrating 5-cc. portions of the sample (diluted with 20 cc. of distilled water) with silver nitrate solution, dichlorofluorescein being used as indicator (5). Values in excess of 0.13% were regarded as abnormal. The pH values were determined electrometrically with the quinhydrone electrode, a value of 6.8 being taken as the upper limit for normal milk*.

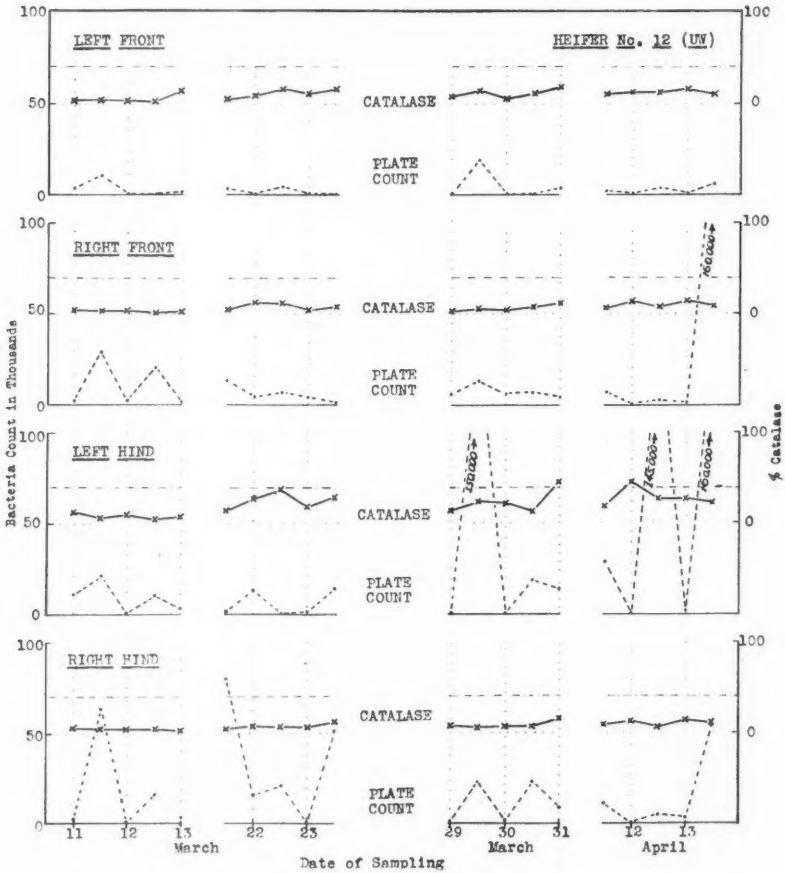

FIG. 4. *Data on foremilk samples, Heifer No. 12, (U.W.)*

* *These limiting chloride, catalase, and pH values for normal milk were based upon previous studies (4) where in the majority of cases these values were found to be substantially equivalent.*

Findings

The data for each quarter are presented in Figs. 5 to 8. It will be observed that the differences in values between foremilk and strippings were most pronounced in the case of catalase; chloride values showed the least differences, while pH values showed slightly greater differences than chlorides. Differences of similar magnitude have never been noted in the few other animals in which a study of both foremilk and strippings samples has been made (4); except for catalase values, which were sometimes slightly higher, both normal and abnormal quarters nearly always showed lower values for strippings.

While all four quarters showed this difference, it was less marked in the right hind quarter, which also showed a lower general level of values by all three tests. It will be recalled that this quarter was producing about 20% more milk than the left hind quarter.

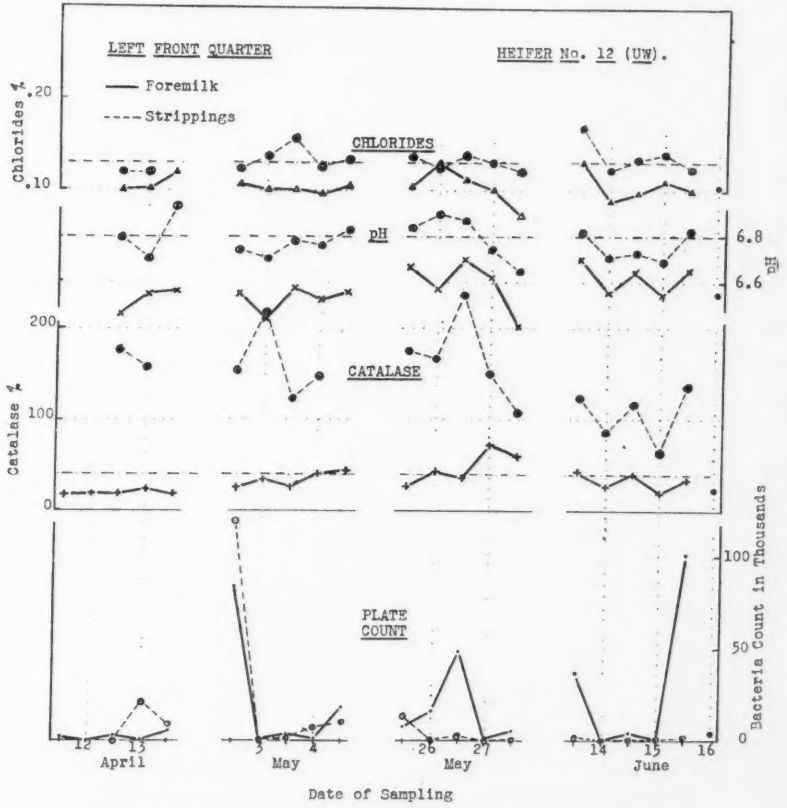

FIG. 5. *Data on foremilk and strippings samples, Heifer No. 12 (U.W.)*

In order to obtain some indication of whether the values for foremilk or strippings were more truly representative of the entire quantity of milk secreted by any one quarter at a given milking, samples of the latter were secured at the afternoon milking of June 16 when the production per quarter was being determined by the use of the special milking machine. The data obtained are indicated on the figures by solid dots. It will be observed that the values, especially those for catalase, were in good agreement with those for the foremilk, only one value (catalase for the right front quarter) being slightly in excess of the normal limits adopted in these studies.

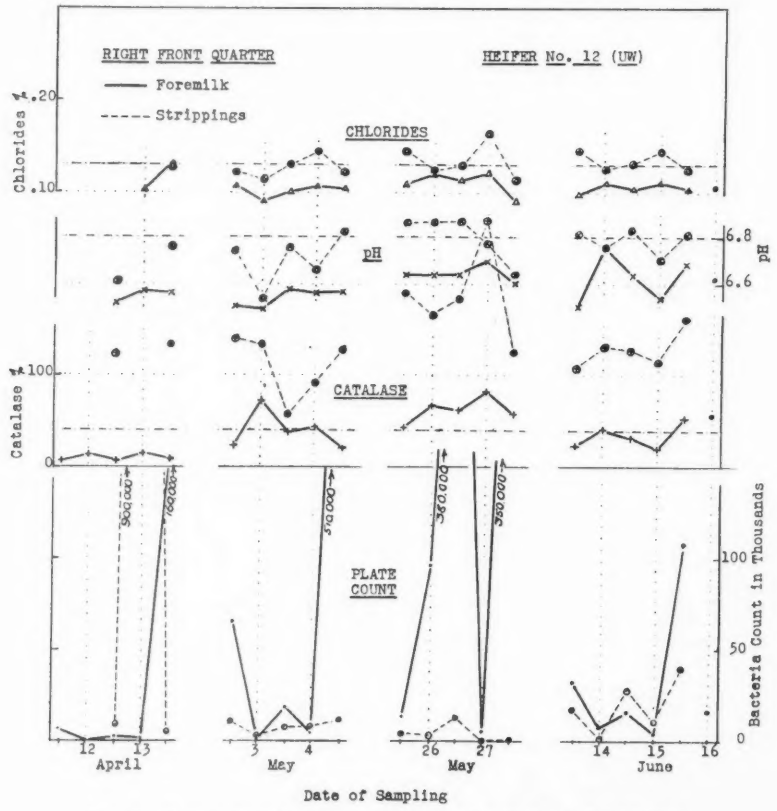

FIG. 6. *Data on foremilk and strippings samples, Heifer No. 12 (U.W.)*

The close agreement in direction and degree of fluctuations in values for chlorides, pH and catalase, which was so striking a feature in the majority of animals previously studied (4), was lacking here. Nor did the values for the various quarters fluctuate together as was often the case, while values for

foremilk and strippings appeared to fluctuate independently and showed little or no relation to fluctuations in bacteria counts. The gradual rise in the levels of values for foremilk up to the period of May 26 to 28 suggested that all four quarters would soon become definitely abnormal, but at the subsequent series of samplings on June 14 to 16 a decline was noted. The departure of the senior author a few days later unfortunately brought these observations to a close. However, routine sampling at the afternoon milking at fortnightly intervals since that time by Dr. Peterson has shown that the general levels of values for chlorides and catalase have increased fairly steadily for all four

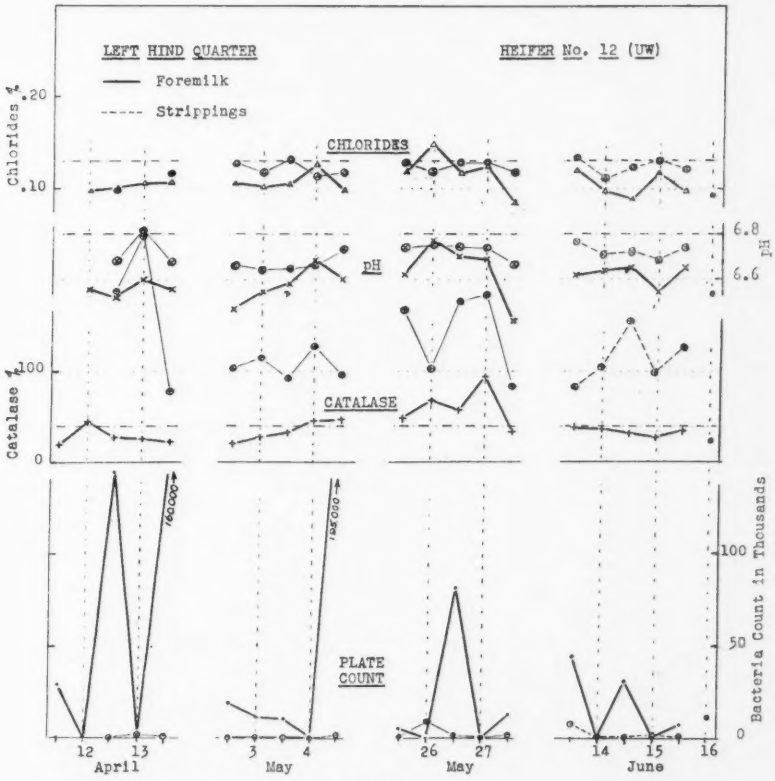

FIG. 7. *Data on foremilk and strippings samples, Heifer No. 12 (U.W.)*

quarters (Table II) so that none could now be regarded as normal. The right front quarter displays the greatest degree of abnormality and the right hind the least. Production for the right hind quarter is over 40% greater than for the left hind, while for the two front quarters production is fairly uniform.

This animal yielded 34.1 lb. of milk on September 6, 1937, seven months after freshening. Bacteria counts from samples taken at the *afternoon* milkings on August 16 and September 13 were fairly low, those for the right front quarter alone exceeding 225 per cc. (4,200 and 3,000 per cc.). This is in line with the findings reported above, high counts being rarely encountered except at the morning milkings.

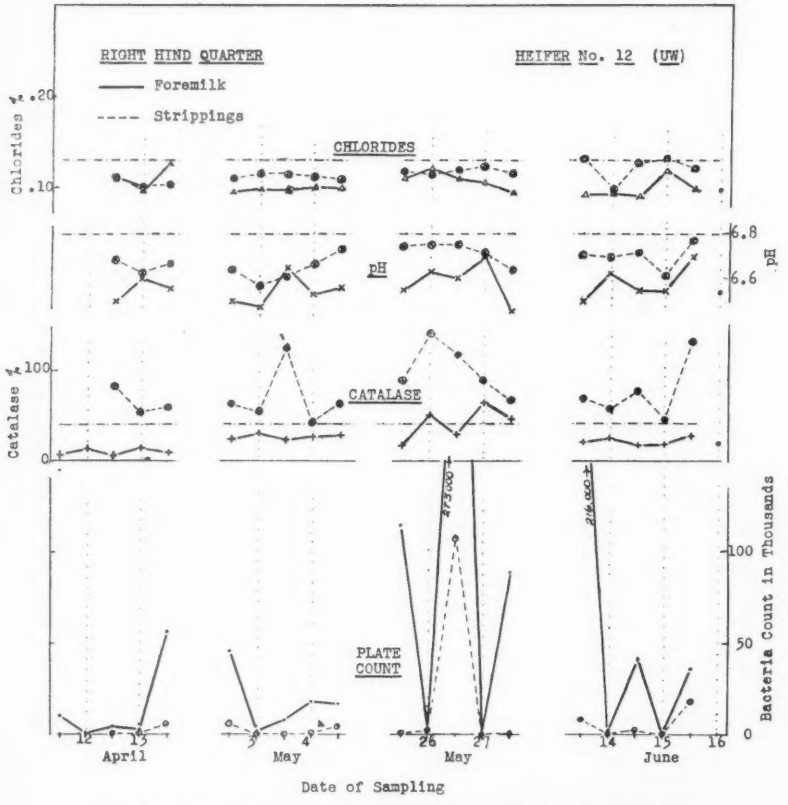

FIG. 8. *Data on foremilk and strippings samples, Heifer No. 12 (U.W.)*

While, in the studies reported in Part I, there was some indication of a correlation between the levels of bacteria counts and of catalase values for the left hind quarter, there was less evidence in this direction from these later studies. This quarter, with no higher general level of counts than the right hind, showed a greater degree of abnormality in secretion, especially in the strippings, while the right front quarter, in spite of a number of extremely high counts and the highest level of counts of the four quarters,

showed only a slightly greater degree of abnormality in the secretion than the left front quarter with a much lower count level. However, the right front quarter later developed a greater degree of abnormality in the secretion, as indicated in Table II.

TABLE II

SUMMARY OF DATA FROM BI-WEEKLY ROUTINE SAMPLING OF FOREMILK OF HEIFER NO. 12 (U.W.) AT AFTERNOON MILKINGS, JUNE 22-SEPTEMBER 13, 1937

	Catalase, %			Chlorides, %			Milk production, average per milking
	Average	Maximum	Minimum	Average	Maximum	Minimum	
Left front	58.4	98	31	0.185	0.200	0.171	8.34
Right front	97.4	126	63	0.200	0.225	0.176	8.14
Left hind	54.8	83	36	0.176	0.187	0.167	8.09
Right hind	42.4	61	26	0.169	0.178	0.153	11.63

General Discussion

With the exception of Heifer No. 12, the data acquired during these studies have not thrown much light upon the point at issue since the majority of the quarters previously regarded as normal have shown no indications of abnormality other than an occasional temporary rise in catalase values. However, Heifer No. 12, which at the start gave some extremely high counts, later became definitely abnormal in all four quarters, suggesting that large numbers of bacteria in the glands may have influenced the secreting tissue sufficiently to change the composition of the secretion.

In the case of the animals reported upon in this paper it is evident that the fairly definite relationship between fluctuations in bacterial numbers and in composition of the secretion which Steck (8) has described in his so-called "latent infection" does not hold. High values for catalase may be encountered along with low counts, as noted for the right hind quarter of Heifer No. 3. On the other hand, extremely high counts may occur with little or no concurrent evidence of abnormality as in the case of Heifer No. 12, although such high counts may be followed by abnormalities in the secretion at a later date. It would indeed be surprising if such enormous numbers of bacteria could be present in the gland for considerable periods without appreciably affecting the composition of the secretion. It must of course be recognized that various factors other than bacterial infection may be responsible for changes in the composition of the secretion; on the other hand, infection may so damage the secreting tissue that abnormal secretion will be noted for some time after the numbers of bacteria have declined to a normal level, as in the case of the right front quarter of Heifer No. 7.

The possibility cannot be disregarded that there may be another biological agent concerned in addition to bacteria; where the bacteria alone are present, the composition of the secretion is scarcely influenced, but when the hypothetical second agent is present, definite changes occur. Although no information

has been obtained to date in these studies which casts any light upon the possible nature of this second agent, its presence would serve to explain such inconsistencies as have been mentioned in the preceding paragraph.

In the present studies no definite relation appeared to exist between yield and composition of milk. Some quarters showing slightly higher levels of catalase values also yielded significantly less milk than the corresponding quarter, while others failed to show any difference. This does not alter the fact that in the case of definitely abnormal quarters, both yield and composition of the milk are often markedly affected (1).

The tremendous fluctuations in bacteria counts, frequently of a regular rhythmic nature, are of interest. Had sampling been confined to the afternoon milkings, the extremely high counts from certain quarters would have been missed almost entirely.

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THE INTERRELATION OF LIPIDS IN THE BLOOD OF NORMAL RABBITS[1]

By Eldon M. Boyd[2]

Abstract

A differential lipid analysis was performed by oxidative micromethods on heparinized plasma and red blood cells of 89 normal healthy rabbits and the normal means and range of blood lipid values established. Within this normal range, the amounts of all lipids in plasma were found to increase at the same relative rate, but a similar relation was found only for phospholipid and cholesterol in the red blood cells. Sex, body weight, blood hemoglobin and season were found to be without effect on the average lipid content of the blood of normal rabbits.

In a previous contribution to this Journal (5), a study was reported of the relation between the amounts of the various lipids in human blood to increasing values of total lipid. In that paper, reference to literature bearing upon the subject was made. Briefly, it was recorded that within the range of normal values the concentration of all lipids in the blood plasma of man tended to increase at about the same rate whenever an increase occurred in total lipid, but that either above or below the normal range the concentration of certain lipids increased or decreased more rapidly than that of others. There was less correlation in amounts of lipids in the red and white blood cells than in plasma.

An investigation was later made, with rabbits as experimental animals, to study the relation between the lipid content of blood and the immunity reactions developed during vaccination or infection. In rabbits vaccinated against *Streptococcus viridans* no significant variations were encountered in the phospholipid or free cholesterol contents of plasma (7). One of the difficulties encountered in the latter work and noted in the results summarized in Table I of that report (7), was the apparently marked variation in the phospholipid and free cholesterol contents of plasma in 28 normal rabbits used as controls.

Rabbits have been extensively used in research on the physiology of lipids, but a review of the literature revealed that extensive studies of variations in the normal amounts of lipids in blood of rabbits have been very few in number. Of the more recent reports, Starup (9) has given values for four lipids—total lipid, neutral fat, total cholesterol and phospholipid—in the blood of 54 normal rabbits. Boyd (4) summarized values for seven lipids in the plasma of 10 normal rabbits used as controls. The lipid content of the blood plasma of rabbits is considerably lower than that of dogs or of man but it is within the same range as that found in another herbivorous animal, the guinea pig (6). When compared with man or dogs, certain enigmatous variations are encountered in the lipid metabolism of rabbits. For example, it is difficult, if not

[1] *Manuscript received December 3, 1937.*
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impossible, to produce a post-prandial lipaemia in this species by feeding fat (1). These species variations must be borne in mind whenever rabbits, and probably other rodents, are used in the investigation of problems in the physiology or pathology of lipids.

The results reported in the present paper were anticipated to yield information on three points, first the range of blood lipid values in normal healthy rabbits, second the relation between the amounts of the various lipids and changes in the amount of total lipid, and third, an adjudication of the reputed importance of certain factors on the concentration of blood lipids. A total of 89 rabbits was used, 15 being females and the rest males. All of the animals appeared on examination to be healthy and most of them had been recently obtained from the farms. They varied in weight from 1,900 to 3,000 gm. They were housed in individual cages in well ventilated and uniformly heated quarters and were fed on a cereal mixture, containing the necessary foodstuffs and vitamins, supplemented by green vegetables from time to time.

Blood for lipid analysis was withdrawn without anaesthesia from the marginal ear veins of animals fasted over night and collected in flasks containing heparin as an anticoagulant. Extracts of plasma and of the red blood cells were made and analyzed by oxidative micromethods as used in previous studies.

Results

Normal Values

In Table I the lipid values obtained in the blood of this group of normal rabbits have been summarized. The significance of each value for each lipid in Table I and the derivation of the several values are the same as those given in Table III of the former paper on interrelations in man (5). The coefficient of variation listed in Table I is the standard deviation expressed as a percentage of the mean.

The blood *plasma* of normal rabbits was found to contain about one-half as much lipid per 100 cc. as the plasma of man. There was somewhat more than one-half as much neutral fat and less than one-half ester cholesterol and total cholesterol. The relative variations of lipid values of plasma were two to three times as great in rabbits as the corresponding variations in man. Reduced to units by comparing corresponding coefficients of variation, total lipid of rabbit plasma was 164% more variable in amount, neutral fat 14%, phospholipid 147%, free cholesterol 247% and ester cholesterol 126% more variable than in man (5). Undoubtedly a good deal of this variation is due to the smaller amounts of lipids in the plasma of rabbits. In other words, a variation of the same absolute amount of lipid would produce a greater change relatively in rabbit plasma than in human plasma. This partial interpretation is substantiated by the fact that the amount of neutral fat in the plasma of rabbits was nearer to that in human plasma than was the amount of any other lipid, and the coefficient of variation for the plasma neutral fat in rabbits was practically the same as that in man, this being the only coefficient not indicating considerably greater variation in rabbits than in man.

These results indicate that in comparing the effects of any experimental manipulation on the plasma lipid content of one group of rabbits with that of another group, a difference of practically 100% or more will be necessary before significance can be claimed for the difference. A change of this magnitude would probably always be pathological, so that the rabbit is not a very suitable animal for purposes of demonstrating lesser physiological differences between groups. This obvious restriction of the use of rabbits in research on the physiology of lipids is not meant to apply to repeated observations on the same animal. The significance of results from the latter type of experiment would depend upon other factors.

The lipid content of the *red blood cells* of normal rabbits (Table I) was found to be slightly lower on the average but not significantly lower than that of human erythrocytes (5). It is of interest that while the lipid content of plasma may differ greatly, that of the red blood cells remains almost identical in rabbits, man (5), guinea pigs (6) and dogs (2). Relative variation in the lipid content of the red blood cells was found practically the same in normal rabbits as in normal man.

TABLE I

THE LIPID CONTENT OF BLOOD IN NORMAL RABBITS

(Results expressed in mg. per 100 cc.)

Value	Total lipid	Composition of total lipid					
		Neutral fat	Total fatty acids	Cholesterol			Phospholipid
				Total	Ester	Free	
Plasma—89 samples							
Minimum	81	0	42	13	0	4	17
Maximum	482	231	311	100	54	59	181
Mean	243	105	169	45	23	22	78
Standard deviation	89	50	66	18	12	13	33
Coefficient of variation	37	48	39	40	52	59	42
Range of ⅔ of values	154–332	55–155	103–235	27–63	11–35	9–35	45–111
Range of 95% of values	65–421	5–205	37–301	9–81	0–47	0–48	12–144
Red blood cells—67 samples							
Minimum	317	0	140	74	0	74	180
Maximum	646	211	411	150	36	136	358
Mean	433	63	251	112	5	107	259
Standard deviation	66	56	57	16	9	15	35
Coefficient of variation	15	89	23	14	180	14	13
Range of ⅔ of values	367–499	7–119	194–308	96–128	0–14	92–122	224–294
Range of 95% of values	301–565	0–175	137–365	80–144	0–23	77–137	189–329

Interrelation of Normal Values

Results in the 89 complete analyses of *plasma* summarized in Table I were divided into 10 groups containing four to fourteen complete analyses each, and arranged according to the value of the total lipid content. Group 1, for example, contained five complete analyses in which the total lipid content was below 125 mg. per cent; Group 2 contained seven complete analyses with total lipid values between 125 and 150 mg. per cent, and so on. The mean value of each lipid in each of these groups was then calculated and the means of the component lipids compared with the means for total lipid. As previously shown for human plasma (5), and as also found herein, there is not a significant difference between results in any two adjacent groups. But the coefficient of correlation between any lipid of plasma and the total lipid values shows that a significant correlation does exist between increasing values for the component lipid and those of total lipid.

The means of the component lipids have been plotted against the means of total lipid of plasma in Fig. 1. The abscissa of Fig. 1 is scaled arithmetically and represents the total lipid values. The ordinate is graduated in logarithms of the base 10 and on it the component lipid values are placed. Using an arithlog ruling in this manner permitted a more immediate visualization of the comparative relative changes in component lipid values with change in total lipid. It is obvious from Fig. 1 that with increase in total lipid of these *normal* rabbits, practically the same relative increase occurred in the amount of all of the component lipids. This substantiates the conclusion drawn from studies in human blood in which also about the same relative increases in the amount of component lipids occurred with increase in total lipid within the *normal* range (5).

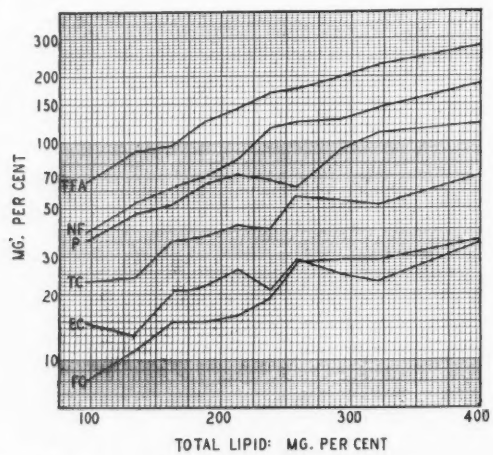

FIG. 1. *Arithlog plotting of changes in the mean values of lipids of blood plasma of normal rabbits with increase in the total lipid. TFA = total fatty acids; NF = neutral fat; TC = total cholesterol; EC = ester cholesterol; FC = free cholesterol; P = phospholipid.*

The lipids of the *red blood cells* showed less correlation when similarly arranged and plotted in Fig. 2. The amount of phospholipid, total cholesterol and free cholesterol increased at about the same proportional rate with increase in total lipid. The amount of total fatty acid increased at a somewhat faster rate owing to a fairly considerable increase in the mean value of neutral fat. There is relatively little neutral fat in most samples of red blood cells and the amount is extremely variable, so that actually there was not a significant increase in the amount of neutral fat with increase in total lipid. Fig. 2 demonstrates clearly the variable nature of the concentration of neutral fat in the red cells, and also of ester cholesterol, of which there is usually very little and most often none at all. It may be concluded that increase in the total lipid of the red blood cells of normal rabbits is accompanied by proportionate increases in phospholipid and cholesterol and occasionally by a marked increase in neutral fat with very little cholesterol ester present at any time. These relationships are identical with those described previously in human red blood cells (5).

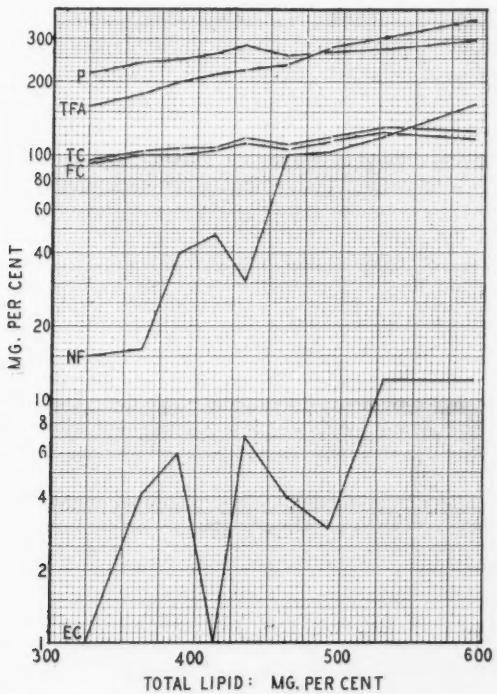

FIG. 2. *Arithlog plotting of changes in the mean values of lipids of the red blood cells of normal rabbits with increase in total lipid. TFA = total fatty acids; NF = neutral fat; TC = total cholesterol; EC = ester cholesterol; FC = free cholesterol; P = phospholipid.*

Certain Factors Supposed to Affect Blood Lipid Values

Sex. The results were grouped according to the sex of the animal and a statistical analysis made of the two groups. There were found no significant differences in the lipid values between the two sexes. On the average, female rabbits contained 22% more phospholipid, 24% more ester cholesterol and 29% more free cholesterol in plasma than did the males. These average differences were not sufficiently great to be statistically significant.

Body weight. The analyses were divided into four groups according to the body weight of the animal, each group having a weight range of 300 gm. Again no statistically significant differences could be ascribed to variations in body weight. The plasma of the lighter and heavier animals contained the least average amounts of lipids. One group of middle-weight animals contained as much as 66% more phospholipid in plasma, but even this difference was insufficient to be significant. A similar difference found in man would likely be significant but the variations were so great in these rabbits that no real difference could be concluded to exist.

Blood hemoglobin. It is a well known fact that anemia is associated with changes in the lipid content of blood in man and in most animals including rabbits (1), although opposite variations are not seen in the antithetical condition of polycythemia (3). The hemoglobin content of the blood of these normal rabbits varied from 63 to 80% (Sahli) and it appeared possible that variation in hemoglobin concentration within the normal range might account for some differences in blood lipid values. Such was not found to be the case, however, when the analyses were grouped according to the hemoglobin value of the blood of the animal. No significant differences were found between lipid values in the several hemoglobin groups.

Seasonal variation. Blood was analyzed in a group of 12 rabbits in the winter, again in the spring and finally in the summer. The highest average values for plasma lipids were found in the spring and the lowest in the winter, but there was at no time a difference great enough to be significant. Nor was there any consistent increase or decrease in the lipid content of blood in individual animals from season to season. The logical conclusion was that changes in season had no consistent effect on the average blood lipid values of normal rabbits. McEachern and Gilmour (8) found no change in the average value of blood cholesterol and change in season in man.

Conclusion

In conclusion, answers were obtained to the questions initially investigated. The lipid content of blood plasma and of the red blood cells of a large group of normal healthy rabbits was determined, the plasma values found to be about one-half as great as those in man and two to three times as variable, and the amounts of lipids in the red blood cells were shown to be similar in all respects to those in the red blood cells of man. Secondly, the same relative increase in the amount of all lipids of plasma was found to occur with increase in total lipid of plasma in normal rabbits, and like results were obtained in the red

blood cells, except that the increases in neutral fat and cholesterol esters were erratic, both relationships being analogous to those seen in normal human subjects. Thirdly, no relation could be established between blood lipid values in normal rabbits and variations in body weight, or the concentration of blood hemoglobin, or the season of the year, or between the two sexes.

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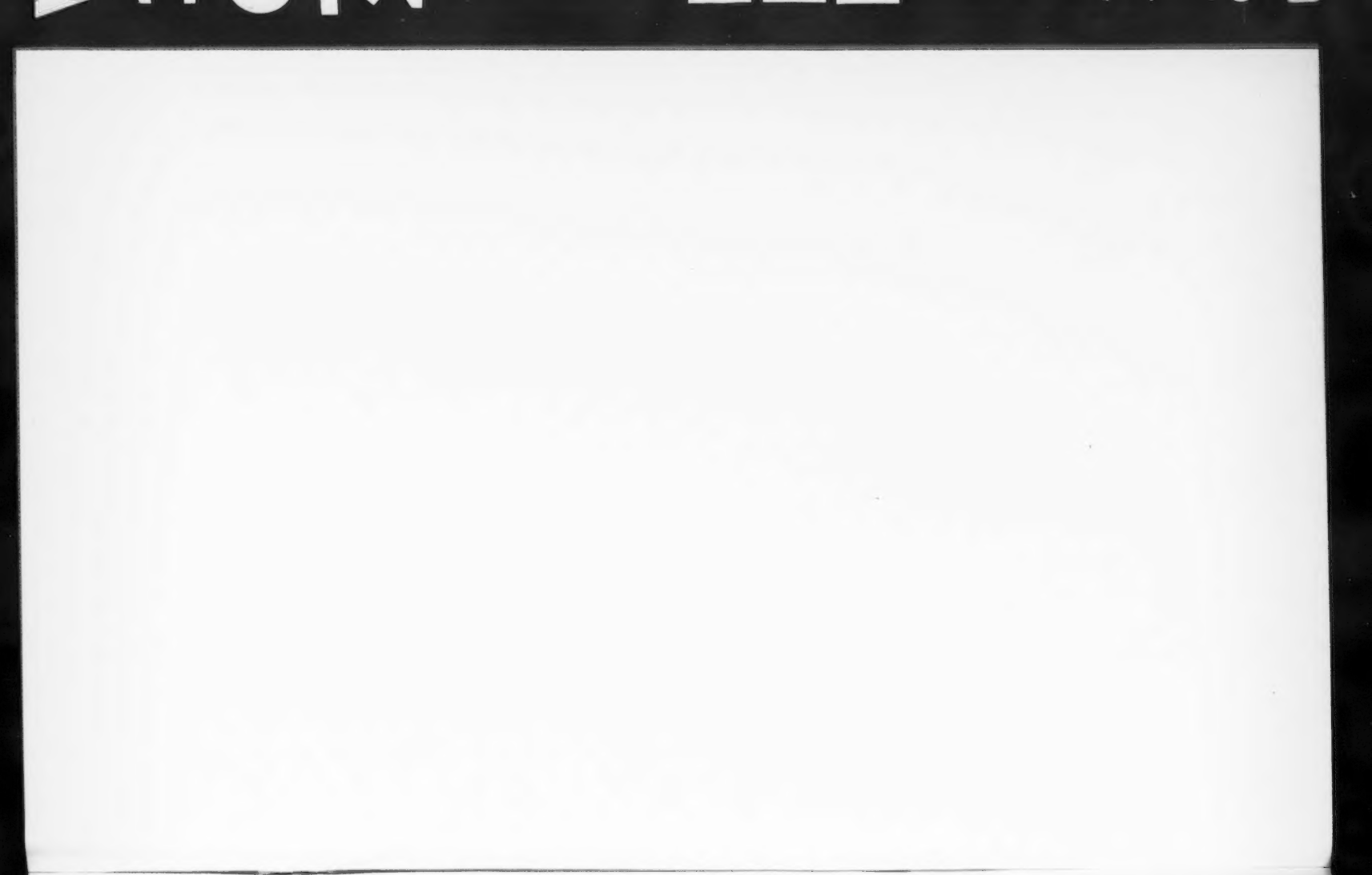
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